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SCIENCE REPORTS OF THE TÔHOKU UNIVERSITY, 4TH SER.
(BIOLOGY), VOL. XVIII, NO. 1, 1947.

ON A NEW SPECIES OF FRESH-WATER POLYP FROM JAPAN

BY

TAKEO ITÔ

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(With 4 Text-figures)

(Received June 15, 1946)

It was in October of 1942 that this kind of fresh-water polyp was collected by me for the first time from a small pond of the Biological Institute in Sendai. They quite diminished in the winter season of the same year, but in April of 1943, appeared again very abundantly in the same place, and they were found in fairly flourish condition in the latter parts of August. In about the same season, some other living specimens were brought to my laboratory from Tokotan near Akkeshi, Hokkaidô, and were cultured continuously. In the early parts of November, many specimens similar to those from the above pond were also collected from Kawato-numa near Sendai, and they were found to be almost sexually mature. Soon after the above collection, many sexual individuals were abundantly found in both of the cultures and pond thereafter.

Thus using these specimens from the above-mentioned three localities, many characteristics shown in the sexual and asexual stages were able to be compared and determined.

Although KUWABARA (1933) described a species from Hokkaidô as identical with *Hydra circumcincta* P. SCHULZE of Europe, it is identical with the present species and seems apparently to be a distinct species new to science. I have given the name of *Hydra parva* to this species from the characteristic being of small size.

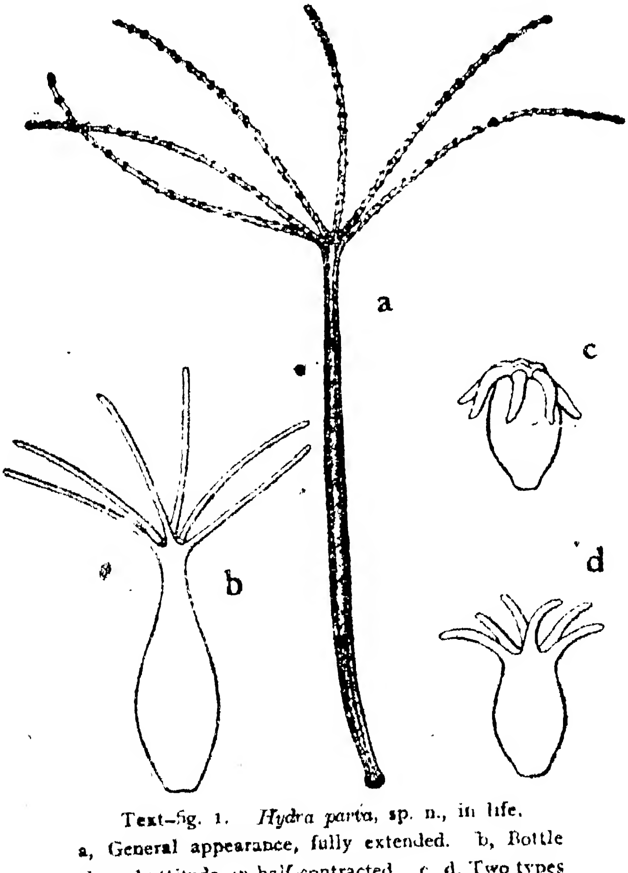
Here I express my hearty thanks to Prof. Dr. SANJI HÔZAWA for his kind guidance and to Mr. MASUTARO KUWABARA for his generosity

expressed to the writer in giving valuable specimens.

Hydra parva, sp. nov.

Column: Small in size, 3–8 mm. usually about 5 mm. in length, when observed in fully extended attitude in life; not clearly differentiated into distal body and proximal stalk; appearing rather bottle-shaped in half-contracted state.

Tentacles: 5–8, usually 6 in number; extended in rather an erect status arching somewhat in the middle portion; always shorter than column, being about 1/3–2/3 length of the latter.



Text-fig. 1. *Hydra parva*, sp. n., in life. a, General appearance, fully extended. b, Bottle shaped attitude, in half-contracted. c, d, Two types of fully contracted manner. (×13).

Colour: Commonly pale pinkish- or reddish brown, frequently marked with clear tint in the region of hypostome.

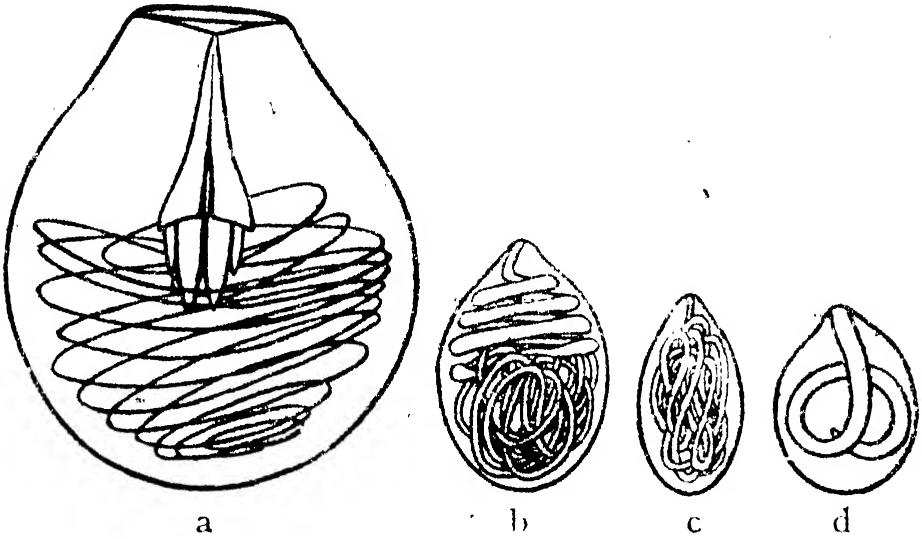
The pattern of tentacle formation in buds: The protuberances arise simultaneously, and thus the tentacles are of equal length throughout their growing stages.

Nematocysts: Usually of four types:

a) Penetrants; 9.0 – 19.0 μ long and 7.5 – 17.0 μ

wide, broadly pear-shaped, with one pole broadly truncated and the other almost rounded.

b) Streptoline glutinants: 7.5–9.5μ long and 5.0-6.5μ wide, oval-shaped being narrowed and pointed at one end, the thread occupying the upper half of the interior of capsule is transversely or obliquely coiled three or four times and shows strong light refraction, while the lower half is wound entangly forming a number of longitudinal loops.



Text-fig. 2. Nematocysts of *Hydra parva*, sp. n a, Penetrant. b, Streptoline glutinant. c, Stereoline glutinant. d, Volvent. (×3600).

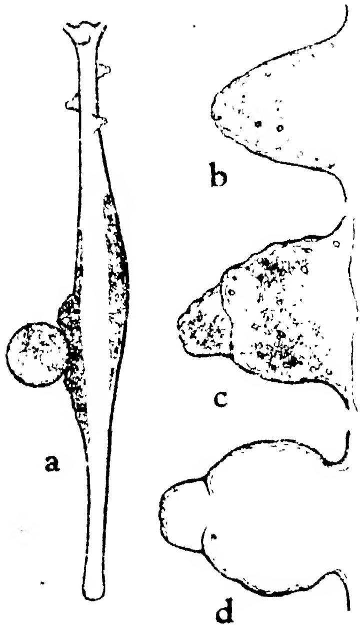
c) Stereoline glutinants: 6.0-8.0μ long and 3.0-4.0μ wide, narrowly oval-shaped.

d) Volvents: 6.0-8.0μ long and 4.0-5.5μ wide, small pyriform.

Sexual reproduction: Hermaphroditic, provided with distal testes and proximal ovaries, rather protandrous; sexually matured in middle and late autumn in nature.

A) Testes: 2–6 usually 3-4 in number occurring in each individual; stumpy conical, rather pyriform in contracted state, with large stocky nipple at apex in which are found numerous active spermatozoa, when matured.

B) Ovaries and eggs: The ovaries are usually swollen out near the budding zone, tinged with pinkish white; the protruded eggs are spherical in form and they fall down from the body of parent after fertilization, and the fertilized eggs are flattened by forming the thecae fastened to the submerged substances.



Text-fig. 3. *Hydra parva*, sp. n. a, Sexual individual with three testes, one ovary and an extruded egg. (Tentacles omitted). b, Young testis. c, Matured testis with a large nipple. d, Ditto, contracted. (a, × 13; b–d, × 130).

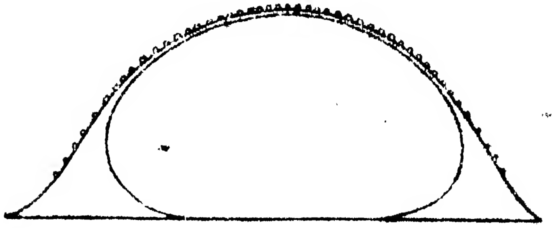
C) Embryonic thecae Thin walled, coloured orange- or reddish brown; helmet-shaped, with many short spines; 0.58 mm. in longest diameter and 0.24 mm. in height, on average.

Habitat: Individuals are found in ponds, marshes and lakes, usually attached to the under side of submerged objects, showing rather negative phototaxis.

Type locality: A small pond of the Biological Institute, Tôhoku University, Sendai.

Other localities: Tôkyô (KUWABARA); Kawato-numa, near Sendai; Kogawara-numa, Aomori Prefecture; Tokotan near Akkeshi, Hokkaidô.

Remarks: The present new species indicates very close relation to *Hydra circumcincta* P. SCHULZE, but the shape of testis and structure of the embryonic theca are difinitely different in these two species in question. *Hydra utahensis* HYMAN, with the similar helmet-shaped embryonic theca, also differs from the present species in the



Text-fig. 4. *Hydra parva*, sp. n. Side-view of the thecated embryo. (× 100).

quite spineless theca and also in some other features.

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(BIOLOGY), VOL. XVIII, No. 1, 1947.

A NEW FRESH-WATER POLYP, *HYDRA MAGNIPAPILLATA*, N. SP. FROM JAPAN

BY

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(With 3 Text-figures)

(Received June 15, 1946)

In June of 1943, the writer collected some individuals of fresh-water polyp from the moats in Matsue, Shimane Prefecture, and also in August of the same year, from Tombetsu-numa, Hokkaido. These specimens produced the sexual organs some time after they were cultured. Of these specimens the gonads and embryonic thecae were quite peculiar differing from those of the previously known species from Japan.

Lately, a great number of specimens were obtained from Miyato-shima, Miyagi Prefecture, being found fairly abundantly in the small reservoirs and rice-fields, in June of 1944. These have showed both of the sexual and asexual reproductions occurring simultaneously, and the other characteristics shown by the same were entirely identical with those of the materials taken from the above-mentioned two localities. It came to light that these animals which came from Matsue, Tombetsu-numa and Miyato-shima are of the same species, which seems to be new to science.

The present paper deals with this new species, named *Hydra magnipapillata* on account of the very large nipple-shaped structure found in each of the testes.

The writer wishes to express his hearty thanks to Prof. Dr. SANJI HÔZAWA for his constant guidance.

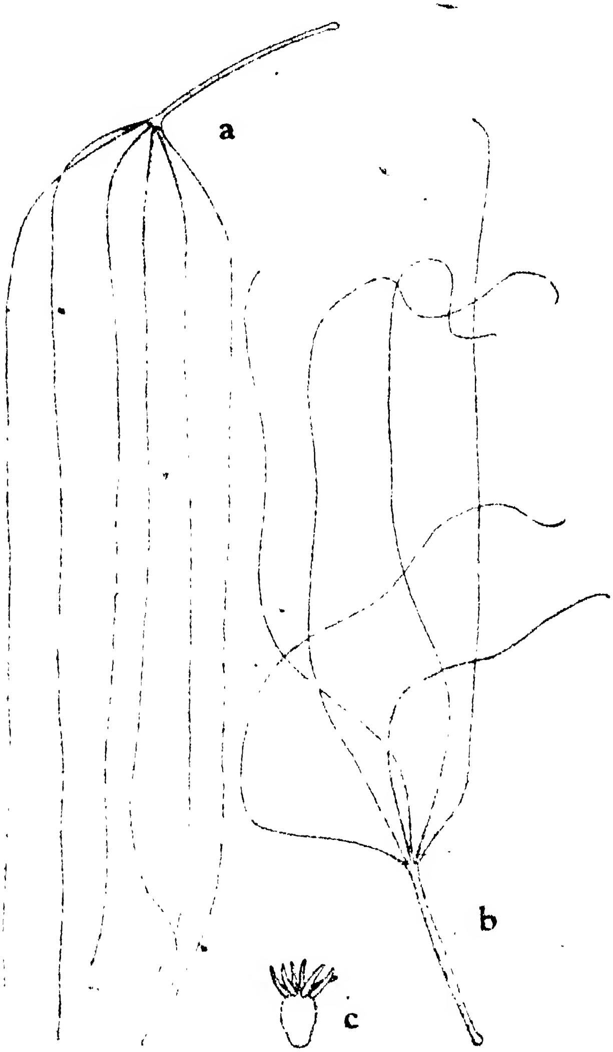
Hydra magnipapillata, n. sp.

Column: Comparatively large, 10-17 mm. long, in fully extended attitude, but rather small in size when grown in some slightly salty habitats. It is cylindrical in form, without any differentiation into a stout body and a slender stalk.

Tentacles : Very long, usually 3-5 and sometimes 6 times as long as column ; highly delicate having compact nematocyst clusters ; 5-8, usually 5 or 6 in number.

Colour : Light brown in general, but sometimes dark- or blackish brown from the engorged food ; greenish brown and green from the symbiotic algae.

Nematocysts : Always of four types :
a) Penetrants : 9.0 19.5 μ long, 6.5-16.5 μ wide, broadly pear-shaped, slightly pointed at round pole.
b) Streptoline glutinants : 10.0-13.0 μ, mostly 12 μ in length, 4.0-6.0 μ wide, elongated oval-shaped, pointed at one end, or rather sole-shaped in longitudinal section ;

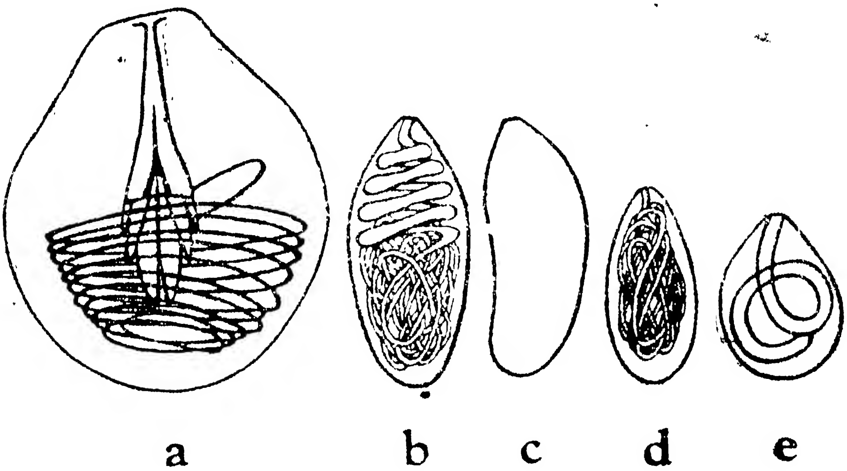


Text-fig. 1. *Hydra magnipapillata*, n. sp., in life. a, Suspended and b, fishing attitudes, when fully extended. c, Strongly contracted state. (×2).

the thread occupying the upper half of the capsule coiled transversely about four times, and represents strong light refraction, while the remaining lower half is entangled.

c) Stereoline glutinants: 7.5–10.0 μ, mostly 9 μ in length, 3.5–4.5 μ wide, narrowly oval-shaped.

d) Volvents: 7.0–9.0 μ, mostly 8 μ in length, 5.0–6.0 μ wide, pyriform.



Text-fig. 2 Nematocysts of *Hyd. magnipapillata*, n. sp.
a, Penetrant. b, c, Streptoline glutinants. (In the case of c, omitted the thread). d, Stereoline glutinant. e, Volvent. (×3000).

The pattern of tentacle formation in buds: They are not produced simultaneously; the first two tentacles arise in pair at the position of figures 4 and 8 o'clock; the next three arise simultaneously at 2, 10 and 6, but sometimes the former two slightly faster than the latter one or in the reverse order of it; the last one at 12; in the individuals provided with seven or eight tentacles it occurs at 1 or 11.

Sexual reproduction: Dioecious; hitherto it was found in spring and summer in the field, frequently in company with budding.

a) Testes: Large, conical or dome-shaped, each being provided with a very large nipple at apex when it is mature. They are produced several to ten in number on each individual, occurring separately not forming any continuous long and low ridge, and being found in the region lying between the hypostome and budding zone.

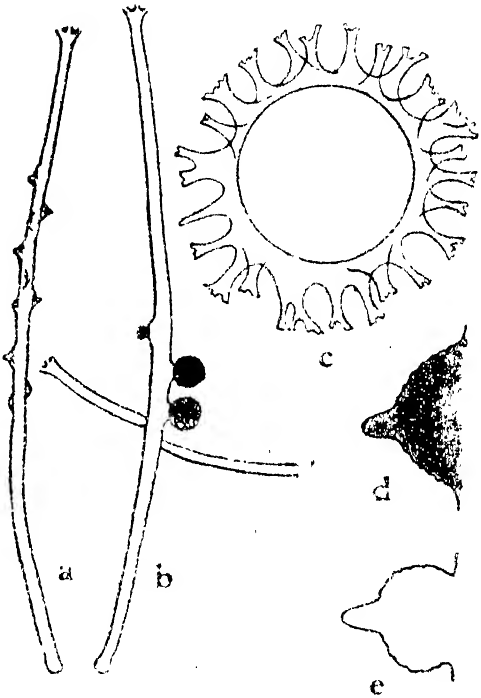
b) Ovaries and eggs: 1–4 in number on each individual, growing in the area situated rather higher than the budding zone.

c) Embryonic thecae. Spherical, 0.45–0.60 mm long in diameter, with many long spines divided into from two to several branches at the tip; their outer surface is covered with mucous membrane, and the embryos are attached to the body of the parent for some time even after the formation of thecae.

Habitat: Reservoirs, rice-fields, moats and marshes, growing attached to the water-plants and to other submerged substances. Individuals endure the rather low pH and the slightly salt-containing medium, and show pretty strong positive phototaxis.

Type locality: The small reservoir at Miyato-shima, Miyagi Prefecture.

Other localities: The moats in Matsue, Shimane Prefecture; Tombetsu-numa, Hokkaidô.



Text-fig. 3. Sexual organs of *Hydra magnipapillata*, n. sp. a, Male, with matured testes. b, Female, with two thecated embryos, a residual cushion after the fall of embryo and two buds grown simultaneously. c, Thecated embryo. d, Matured testis, with large nipple. e, Ditto, contracted. (a, b, Tentacles omitted). (a, b, ×8; c, ×80; d, e, ×60.

Remarks: This new species closely resembles *Hydra littoralis* HYMAN, but apparently differs from it in the length of tentacles, in the pattern of tentacle formation in buds, in the size of nematocysts, in the mode of development of testes and lastly in the habitat. *H. littoralis* is found in the moving or flowing water as its specific name indicates, while the present species is always found in stagnant water. The present species also differs from *Hydra vulgaris* PALLAS in the length of tentacles, form of streptoline glutinants, shape of testes and in the sexuality.

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DESCRIPTION OF A NEW *PELMATOHYDRA* FROM JAPAN

BY

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(With 4 Text-figures)

(Received June 20, 1946)

In the autumn of 1942, in a small pond of the Biological Institute, I found some specimens of a fresh-water polyp closely allied to *Pelmatohydra oligactis* (PALLAS). In the August of 1943, some living specimens of the same features as those above-mentioned, were collected by Mr. M. KUWABARA, in Sapporo, Hokkaidô and were sent to the present writer by his courtesy. During the season covering July and August of 1944, a large number of individuals of the same kind of fresh-water polyp were also collected from the stream near Shimekiri-numa and from the spring of Yokoyama-Fudôson, Miyagi Prefecture. Further more, in late autumn of the same year, a number of the localities of this kind of animal were added by the subsequent collections tried in Kogawara-numa, Aomori Prefecture and in the marshes near Oiwake, Akita Prefecture.

These materials obtained from the above-mentioned localities were partly fixed, and the remaining part was cultured long time continuously in order to observe the life history and the mode of sexual reproduction. Thus at present I have made clear the specific characteristics in detail excepting the structure of embryonic theca.

In the present paper I should like to give the description of this fresh-water polyp which seems to be new to science.

It is my pleasant duty to express my hearty thanks to Prof. Dr. S. HÔZAWA who has kindly guided me. Thanks are also due to Mr. M. KUWABARA for his kindness expressed to me in giving precious specimens.

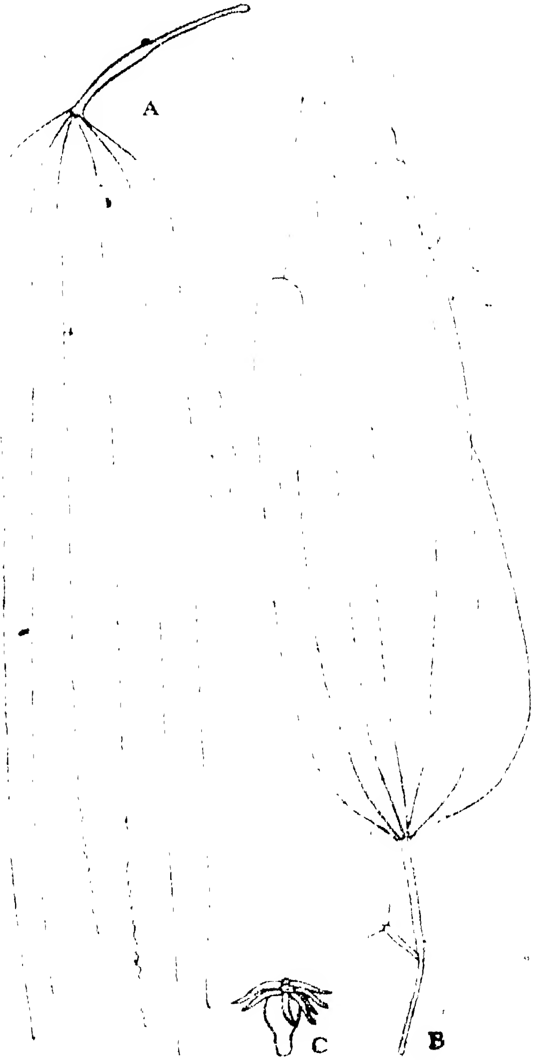
Genus *Pelmatohydra* P. SCHULZE

Pelmatohydra robusta, n. sp.

Column: Very large, being 10–20 mm., usually 15 mm. in length, when measured in healthy and fully stretched state in life, but 3–5 mm. long when contracted; typically being differentiated into stout body and slender stalk from morphological and physiological points of view. It presents rather an elongated wine-glass-shape, the stalk generally 1/3–2/3 of the column in length.

Colour: Clear brown, but sometimes dark-, orange- and reddish brown being effected either by the food or medium of habitat, and sometimes it looks green from the symbiotic algae; the body region is always tinged and the stalk region is pale and fairly transparent.

Tentacles: Typically long, very delicate, 2–4

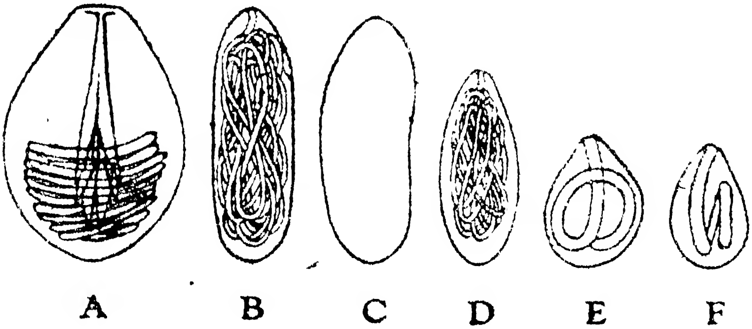


Text-fig. 1. *Pelmatohydra robusta*, n. sp., in life. A, Suspended and B, erect attitudes when fully extended. C, Strongly contracted state. (×2).

times as long as column, but the virtual length are rather longer than those of *Hydra magnipapillata*, in extended state it is of nearly same attitude as the latter; they are 4–10 in number, showing considerable variation by the locality and season, *e. g.*, commonly 7–8 in the case of those grown in the stream near Shimekiri-numa, while they are 5–6 in those which came from other localities.

Nematocysts: Divided into four types:

A) Penetrants: Rather small, showing somewhat narrower variation in size than other species, 9.0–14.0 μ long, almost as long as the streptoline glutinants, and 6.0–11.0 μ wide; broadly pear-shaped, but are more slimmer than those of *H. parva*.



Text-fig. 2. Nematocysts of *Pelmatohydra robusta*, n. sp. A, Penetrant. B and C, Streptoline glutinants. (In C, the thread is omitted.) D, Stereoline glutinant. E, and F, Volvents. (× 3000).

B) Streptoline glutinants: 9.5–12.0 μ, mostly about 10.5 μ in length and 3.5–4.0 μ wide; cylindrical or elongated kidney-shaped, somewhat pointed at one end, with the thread showing some number of longitudinal coils.

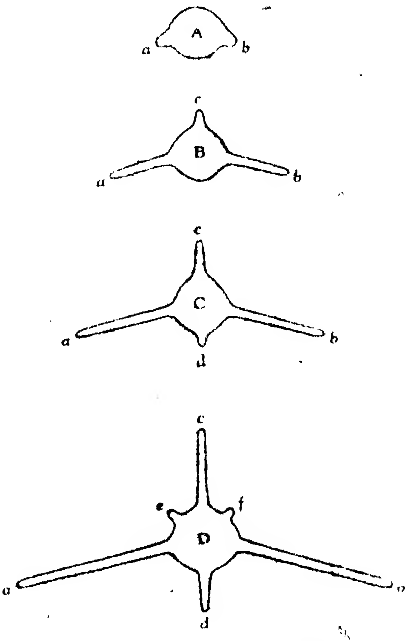
C) Stereoline glutinants: 7.0–9.0 μ long and 3.0–4.0 μ wide; narrowly oval-shaped being pointed at one end.

D) Volvents: pyriform; 5.0–7.0 μ long and 3.5–5.5 μ wide.

The pattern of tentacle formation in buds: Tentacles appear in the particular order, shown in Text-fig. 3. The first two are usually much longer than the others, and these tentacles arisen from the young buds show a remarkable discrepancy in length when they are completed.

Buds: Budding zone is located near the boundary line drawn between the body and stalk; usually 1–5 but sometimes 8 of the buds occur in each individual at the same time.

Sexual reproduction: Strictly dioecious; the gonads are seen always in the body region, but never in the stalk and they are developed either in early winter or in spring in nature; the male or female clons are produced frequently by vigorous asexual reproduction in various habitats.



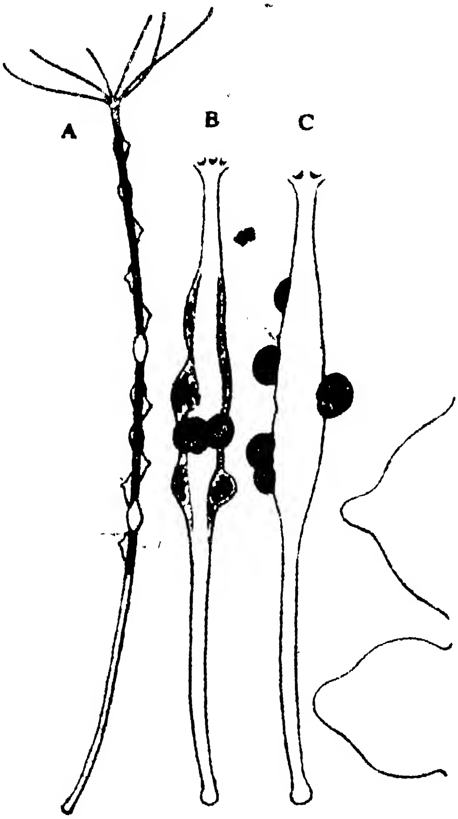
Text-fig. 3. The order of tentacle formation in buds. (A to D, developing stages; a-f, developing tentacles).

A) Testes: Large, rather conical in form, but appearing somewhat pyriform in contracted state. There is found a conspicuous nipple at the tip of each testis when it is mature, as in the case of *H. magnipapillata* and is tinged with yellowish white; from several to 15 of these are developed in one individual; the mature males are rather slender in form and are of the pale tint. And the differentiation into body and stalk becomes almost indistinct and thus the tentacles also become very short in length.

B) Ovaries and eggs: The process of development is almost similar to that of *H. magnipapillata*; several number or nearly so arising in each female.

C) Embryonic thecae: The eggs are frequently produced from the female clons, but hitherto they were seen always collapsed and not fertilized by the sperm owing to the fact that the maturity of the male clons not being attained yet at that time. Thus to my regret the embryonic thecae have not been seen yet of this species.

Habitat: Ponds, marshes, reservoirs and spring, and numerous individuals were also found in the stream of slow current; animals are found attached to the submerged parts of some water-plants and of other objects, showing fairly strong positive phototaxis. They are found alive in quite healthy condition throughout the winter season, differing from other species.



Text-fig. 4. Sexual individuals in life. A, Mature male with many testes. B and C, Females with several ovaries and protruded eggs. (Tentacles omitted). D, Enlarged testis with well developed nipple. E, Ditto, contracted. (A, ×7; B and C, ×6; D and E, ×70).

Type locality: The spring of Yokoyama-Fudôson, Miyagi Prefecture.

Other localities: The pond of the Biological Institute, Sendai; a stream near Shimekiri-numa, Miyagi Prefecture; Kogawara-numa, Aomori Prefecture; some marshes near Oiwake, Akita Prefecture; a pond in Sapporo, Hokkaidô.

Remarks: This new species closely resembles *Pelmatohydra oligactis* (PALLAS), described from Europe and America, in most of the characteristics, differing only in the shape of testes and in some minute features. Namely, the testes occur rather small in number and each is provided with a particularly large nipple, while in the case of *P. oligactis* they are entirely devoid of the nipple and occur very numerously. The problem as regards the structure of embryonic theca which seems to be necessary for the exact identification should be left until we obtain suitable materials.

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TWO NEW SPECIES OF FRESH WATER POLYP FROM JAPAN

BY

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(With 5 text-figures)

(Received July 2, 1946)

(I)

In 1942–1944, some fresh-water polyps which seem to be very common in Japan were collected from widely separated localities. Some of these specimens were cultured in laboratory for a prolonged time and have been observed by the writer, and these polyps were identified as belonging to a distinct species new to science.

Before proceeding further, the writer begs to express here his deep gratitude to Prof. Dr. S HÔZAWA for help rendered him.

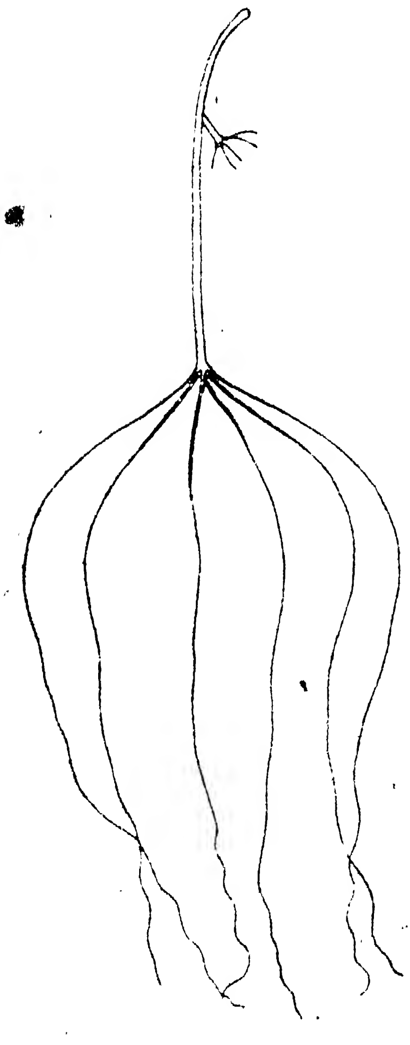
Hydra japonica, n. sp.

Column: Moderate in size, being 5–15 mm. in length, when fully extended in healthy condition. However, the size of column shows variation to a certain degree influenced by some environmental factors such as nutrition, nature of medium, and by the attachment of algae, etc.
It is almost cylindrical in shape, without being differentiated into a stout body and a slender stalk.

Colour: Usually straw-colour or light brown, but sometimes darkish-, greenish-, reddish- and palish brown, and rarely it looks clear green being attached by the minute green algae.

Tentacles: Rather longer than column, being 1.5–2.5 times as long as column in general; in extended appearance it is not so delicate as in *H. magnipapillata*; 4–9, generally 5 or 6 in number, and they sometimes are bi- or trifurcated at the tip.

Buds: Usually 1–3, sometimes 5 of buds arise in each individual at the same time and they occur in rather difinite order.



Text-fig. 1. Fully extended attitude of *H. japonica*, n. sp., from life. (×5).

The pattern of tentacle formation in buds: It resembles closely that of *H. magnipapillata.*

Nematocysts: Animals are provided always with four types:

A) Penetrants: 8–16 μ long and 6–13 μ wide; broadly pear-shaped, slightly pointed at the broader end.

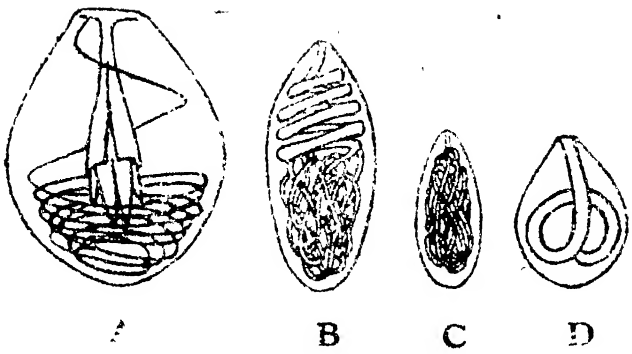
B) Streptoline glutinants: 10–12 μ long and 4–5 μ wide; elongated oval-shaped, pointed at one end, with the thread winding in the same manner as in the case of *H. magnipapillata.*

C) Stereoline glutinants: 6–8 μ long, and are almost as long as the volvents, and 3–4 μ wide; elongated oval-shaped, being pointed at one end.

D) Volvents: 6–7 μ long and 4–5 μ wide; pyriform.

Sexual reproduction: Dioecious in general; the occurrence of gonads was observed in all seasons year round excepting the severe winter time in nature, while it is able to be accelerated by the artificial fluctuation of the temperature and by the volume of foods given in laboratory.

A) Testes: They occur usually several in number in each male, com-



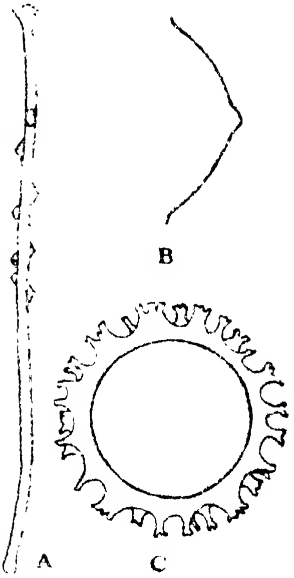
Text-fig. 2. Nematocysts of *H. japonica*, n. sp. A, Penetrant medium size. B, Streptoline glutinant. C, Stereoline glutinant. D, Volvent. (× 3000).

monly being located in the part lying between the budding zone and hypostome, but sometimes being found in rather lower part of the same; moderate in size; conical in form and is provided with a nipple when mature, but the nipple is much smaller and more indistinct than in the case of *H. magnipapillata*.

B) Ovaries and eggs: The ovaries occur generally in the region situated rather higher than the budding zone, but they appear rarely in the lower region of the same; they develop in the same process as in that of *H. magnipapillata*.

C) Embryonic thecae: Clear yellowish brown; spherical in shape, with many spines similar to those of *H. magnipapillata* in shape, but much shorter than the latter; *ca.* 0.5 mm. in diameter of thecae and *ca.* 0.05 mm. in length of spines.

Habitat: Ponds, marshes and lakes etc., namely the animals inhabit in the various sorts of stagnant water being attached to the



Text-fig. 3. Sexual organs of *H. japonica*, n. sp., from life. A, Male with several testes (tentacles omitted). B, Enlarged testis. C, Enlarged embryonic theca. (A, × 8; B, × 70; C, × 60).

water-plants such as *Potamogeton* sp. and others. The number of individuals in those habitats may be fluctuated remarkably by the foods, medium, temperature and other environmental factors, and then they are diminished frequently in very short time after the vigorous increasing.

Type locality A pond near Sendai, Miyagi Pref.

Other localities Lake Ikeda, Kagoshima Pref.; several small ponds near Arao, Kumamoto Pref.; a small pond in Saga, Saga Pref.; two small marshes near Fukuoka, Fukuoka Pref.; a reservoir near Nakatsu, Ôita Pref.; Lake Biwa, Shiga Pref.; several ponds and marshes near Sendai and Shiogama, Miyagi Pref.; Lake Ônuma, and a small pond in Sapporo, Hokkaidô.

Remarks Some zoologists in Japan have frequently confused the present new species with *H. attenuata* or *H. vulgaris* of Europe, hitherto. This new species is closely allied to *H. cauliculata* HYMAN, but the former differs from the latter in the following characters: 1) the form of column; 2) the pattern of tentacle formation in buds; 3) the length of glutinants and volvents; 4) the size and shape of testes. It differs from *H. attenuata* PALLAS, in the shape of streptoline glutinants, in the size of nematocysts and in the pattern of tentacle formation in buds, and also differs from *H. magnipapillata* ITÔ, in the length of tentacles, in the form of testes and in the length of spines of embryonic thecae.

(II)

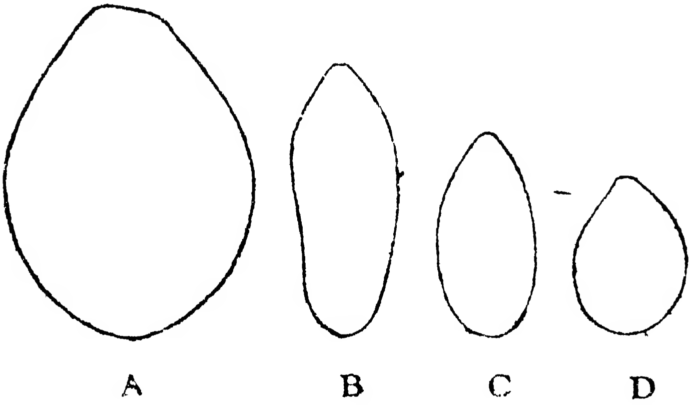
Many specimens of a fresh-water polyp were found in some marshes and ponds near Sendai and Rifu, Miyagi Pref., and also in the moats of Sôma-castle, Fukushima Pref., in autumn and in early winter in 1942 and 1943. At first, they were considered to be identical with *H. japonica* mentioned above, from the close resemblance in the general appearance and in the embryonic thecae. But the examination of nematocysts in detail and the observation of testes have proved that this fresh-water polyp belongs to a distinct species new to science.

Hydra paludicola, n. sp.

Column: Rather large, 8–17 mm. in length, when fully stretched in life; hypostome appears generally flattened conical in shape; almost cylindrical in form, being not differentiated into a stout body and a slender stalk.

Colour: It resembles closely that of *H. japonica*.

Tentacles. Somewhat longer than column, and usually shorter than 2 times of length of the latter; when fully extended it presents rather more erect status than *H. japonica*; 5–11, usually 6 or 7 in number.



Text-fig. 4. Four types of nematocysts of *H. paludicola*, n. sp. (Thread omitted). A, Penetrant. B, Streptoline glutinant. C, Stereoline glutinant. D, Volvent. (× 3000).

Buds: Usually 1–4 in number being produced in each individual simultaneously; the budding zone is located in about one-third of the column length from the pedal disc.

The pattern of tentacle formation in buds. It is the same as that of *H. japonica*.

Nematocysts: Always of four types; and the shape and thread present about the similar form and coiling as that of *H. japonica*.

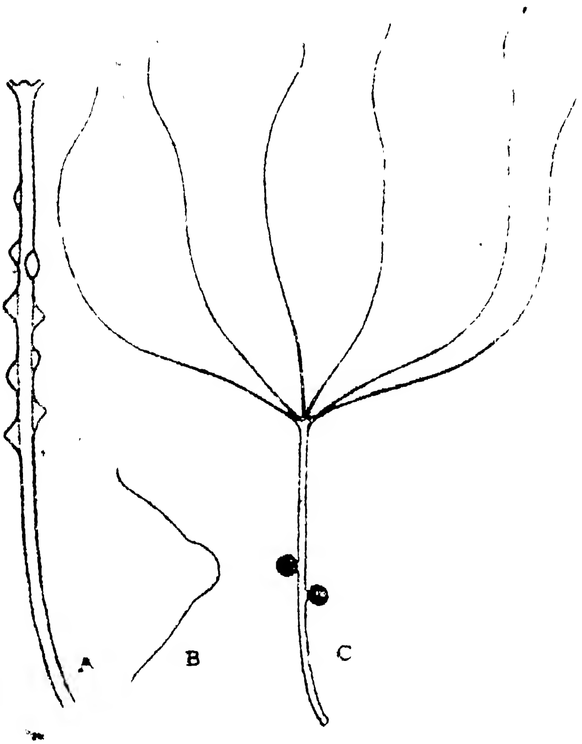
A) Penetrants: 9.0–17.0 μ long and 7.0–13.5 μ wide.

B) Streptoline glutinants: 11.5–13.5 μ long and 4.5–5.5 μ wide rather larger than that of *H. japonica*.

C) Stereoline glutinants: 8.5–9.5 μ long and 4.0–4.5 μ wide, they are about the intermediate in length between streptoline glutinants and volvents.

D) Volvents: 6.5–7.5 μ long and 4.5–5.5 μ wide.

Sexual reproduction Dioecious, it has been observed hitherto in late autumn in nature, occurring sometimes together with asexual reproduction in the same individual.



Text-fig. 5. Sexual Individuals of *H. paludicola*, n. sp., from life. A, Male with several testes (tentacles omitted). B, Enlarged testis. C, Female with two thecated embryos. (A, ×7; B, ×70; C, ×3).

A) Testes: Generally from several to ten in number in each male, being usually developed on the body wall between the hypostome and budding zone; moderate in size; conical in form, with a large, but rather obscure nipple when they reached to the maturity.

B) Ovaries and eggs: 1–3 of eggs are produced in each female in general.

C) Embryonic thecae: Orange brown; spherical in shape, covered with spines same as those of *H. japonica*; thecae are 0.45–0.55 mm. in diameter and spines are 0.045–0.055 mm. in length.

Habitat Marshes, ponds and moats; they were found frequently from some marshes and other stagnant waters with muddy bottom.

Type locality: Two marshes near Rifu, Miyagi Pref.

Other localities: Several marshes and ponds near Sendai, Miyagi Pref.; the moats of Nakamura, Fukushima Pref.

Remarks: This new species seems to be very closely related to *H. japonica*, but the following characteristics of *H. paludicola* will easily distinguish this species from *H. japonica*. First, the stereoline glutinants have a intermediate length between streptoline glutinants and volvents, and are relatively larger than those of *H. japonica* together with streptoline glutinants. Secondaly, the testes have a thick nipple when mature, and then in the whole present a higher and larger conical form than that of *H. japonica*.

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THE LARVA AND POSTLARVAL DEVELOPMENT OF SOME REEF-BUILDING CORALS. I.
POCILLOPORA DAMICORNIS CESPITOSA (DANA)

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(With six Text-figures)

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INTRODUCTION

The investigations made on the larvae and postlarval development of the Madreporaria are comparatively few. As for the corals which belong to the genus *Pocillopora*, although there are some data given by several authors (EDMONDSON, '29; STEPHENSON, '31; HADA, '32; KAWAGUTI, '41), but the behaviors and developmental processes of the larvae and also of young polyps are not yet known in full details.

In the present paper will be given the results of some works made concerning to such subjects as given above The following observations had been carried on at the Palao Tropical Biological Station in the Palao Islands of the former mandated South Sea Islands of Japan during my stay from 1938 to 1942.

Before going further I wish to acknowledge my indebtedness to the Japan Society for the Promotion of Scientific Reseach which by financial aid has rendered it possible for me to carry out the present investigations. To Emer. Prof. Dr. SHINKISHI HATAI, the former director of the station given above, I am indebted for his kind direction throughout the work. Further I wish to express my sincere thanks to Prof. Dr. SANJI HÔZAWA of the Tôhoku Imperial University for his cordial guidance after I left the station.

I. MATERIAL

Pocillopora damicornis cespitosa (DANA) is one of the compound or colonial reef-building corals and is widely distributed in the fringing reefs which are developed around many small islands in the Iwayama Bay of the Palao lagoon. It is commonly found also at the outer margin of the reef flat in the bay. The colonies present a branching form consisting of very small corallites. The colouration of the soft part during life is greenish brown, while the blunt tips of each branchlet are usually pale and more or less reddish. The polyps of the corals extend their tentacles during daytime as was observed on several other species (EGUCHI, '35; STEPHENSON, '40, KAWAGUTI, '40) and when the tentacles are fully lengthened, they look rather green in hue.

In general it is very difficult to obtain the larvae of the most species of Madreporaria. Indeed, I could gain the larvae of only ten species or so from the bay in spite of all efforts to collect them for a long period extending over about four years, while EGUCHI ('38) had already discovered over one hundred of species of corals from the same bay. On the contrary the larvae of present coral are obtained very easily every month throughout the year in a definite period on which I will describe later on. In addition to this, they are of rather large size and are well developed under the laboratory conditions. Thus they proved to be one of the most excellent materials for the studies on the coral larva and its developmental steps.

II. OBSERVATION

All of the observations made on both of the free swimming and postlarval stages were made in the laboratory. For the purpose of obtaining the larvae many colonies, about 290 grams in weight, were placed near the window in glass vessels, each having 30 cm. of diameter and hight. The vessel was filled with sea water and were renewed once every day. The temperature of water ranged from 26°C. to 30°C. throughout the observation period. Under these environmental condi-

TABLE 1. Showing the period of extrusion of planula extending 1938 to 1941 in *Pocillopora damicornis caespitosa* (DANA).

Date of collection	Moon's age	Planula extruded (+), or not extruded (−)	Date of collection	Moon's age	Planula extruded (+), or not extruded (−)
1938			Jun. 9	22	−
Jul. 26	29	+	10	23	−
Aug. 24	29	+	14	27	+
1939			21	5	−
Jan. 18	28	+	23	7	−
19	29	+	24	8	−
21	2	+	26	10	−
Feb. 6	18	−	27	11	−
7	19	−	Jul. 4	18	−
16	28	+	5	19	−
18	30	+	8	22	−
19	1	+	13	27	+
24	6	−	20	4	−
25	7	−	22	6	−
26	8	−	26	10	−
27	9	−	27	11	−
28	10	−	31	15	−
Mar. 7	17	−	Aug. 1	16	−
8	18	−	3	18	−
11	21	−	4	19	−
13	23	−	7	22	+
14	24	−	12	27	+
18	28	+	14	29	+
22	2	+	15	1	+
23	3	+	19	4	+
27	7	−	24	10	−
28	8	−	25	11	−
Apr. 3	14	−	Sep. 1	18	−
7	18	−	2	19	−
8	19	−	4	21	−
11	22	−	5	22	−
12	23	−	8	25	+
22	3	+	9	26	−
May. 4	15	−	14	2	+
5	16	−	15	3	+
9	20	−	16	4	+
10	21	−	19	7	−

	20	8	–		7	8	–
	25	13	–		8	9	–
	26	14	–		9	10	–
	27	15	–		29	1	+
Oct.	2	20	–	Dec.	3	5	+
	3	21	–		28	30	+
	4	22	–	1941			
	6	24	–	Jan.	22	25	+
	11	29	+		25	28	+
1940					28	2	+
Jun.	26	21	–		29	3	+
	27	22	–		30	4	+
Jul.	3	28	+	Feb.	26	1	+
	5	1	+		27	2	+
	7	3	+	Mar.	26	1	+
	8	5	+	May.	3	8	–
	9	6	+		4	9	–
	10	7	+		9	14	–
Aug.	1	28	+		10	15	–
	2	29	+		12	17	–
	3	30	+		13	18	–
	4	1	+		23	28	+
	5	2	+		25	30	+
	6	3	+		26	1	+
Sep.	4	3	+	Jun.	22	28	+
Oct.	6	6	+		23	29	+
Nov.	1	2	+	Jul.	25	2	+
	2	3	+		26	3	+
	6	7	–				

tions the corals were kept alive for several days and extruded a great number of well developed larvae, usually some hundreds or more arising from one colony at a time. Each of the colonies was collected from different habitats during high tide and fresh materials were successively brought to the observasions.

1. *The larva or planula*

(1) THE PERIOD OF EXTRUSION.

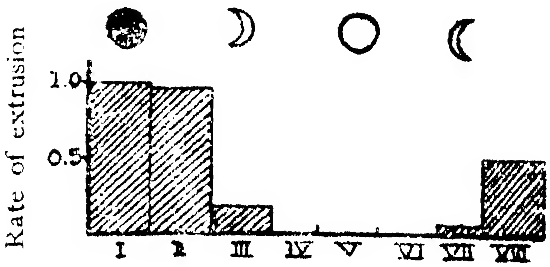
The observation on the period of extrusion had been made for twenty-five months extending over four years and during the lapse of time the liberation of planula took place in twenty-three months (Table 1). As this result it may be confirmed that the planulae are extruded throughout the year as was conjectured by HADA ('32) and MOTODA ('38).

Then, in order to determine the time when the planulae are set free from their parents, all the observable period was divided into nearly equal eight divisions according to the phases of the moon. The first division represents the time of the new moon and the second is the intermediate period between the first and third which corresponds to the time of the first quarter, and so on. The result is shown in Table 2 and in Text-figure 1, from which it will be known that the extrusion of planula took place in the first to third and besides in the seventh and eighth divisions, while it never occurred in the fourth to sixth divisions. In addition to this, the well developed planulae were usually found in the first three divisions, whereas those appeared in the last two divisions were often whitish in colour and of smaller size and seemed to be premature ones spawned by some unnatural stimulus. As already mentioned some hundreds or more of planulae were usually liberated from their parent colony at the same time, but in some cases only a few of them were set free from the colony of

TABLE 2. Showing the relation between the extrusion of planula and the phases of the moon in *Pocillopora damicornis cespitosa* (DANA).

Division	Moon's age	Planula extruded	Planula not extruded	Number of times of observation	Rate of extrusion
I	28 – 1	27	0	27	1,00
II	2 – 5	23	1	24	0,96
III	6 – 9	3	16	19	0.18
IV	10 – 12	0	8	8	0
V	13 – 16	0	10	10	0
VI	17 – 20	0	16	16	0
VII	21 – 23	1	14	15	0.06
VIII	24 – 27	4	4	8	0.50

nearly equal weight. These cases were recorded as negative in the table. From these results it may be ascertained that the planulation takes place every month throughout the year according to the moon's age, that is, it is regularly commenced before the time of the new moon and becomes most active at the time of the new moon or thereabout lasting it for about a week. When it comes to the time of the first quarter, the extrusion is finished and never occur next for about ten days including the time of the full moon. In this way the planulation of the present coral shows the lunar periodicity as in the case of *Fungia* (ABE, '37).



Text-fig. 1. Showing the extrusion of planula in each phase of the moon in *Pocillopora damicornis cespitosa* (DANA).

(2) THE EXTERNAL FEATURES

The planula is capable of changing its body in various forms. When contracted rapidly, it becomes spherical and when expanded, it presents pear shape. The longitudinal axis measures about 2.0 mm. in length and the broadest width of the transverse axis is about 0.5 mm. in its elongated form. The colouration of body is brown due to the symbiotic algae, viz., Zooxanthellae, which are usually found in a great quantity distributed all over the body. Sometimes the planulae are very dark or very light in hue in proportion to the quantity of the algae. So far as I know the planulae of different species of corals present the similar colouration to that of respective parent corals. This fact was also noticed on those of *Balanophyllia* sp. and *Montipora ramosa* (KAWAGUTI, '44).

An oral aperture has already been opened as was found in the case of *Siderastrea* larva (DUERDEN, '02, '04), having a narrow slit-like appearance. The body is covered uniformly with short cilia and its active ciliary movement can be observed under a microscope. The mesenteries appear as dark striations on the ciliated body surface, but none of the tentacles and skeletons is yet developed (Text-fig. 4, a).

The features of planula above mentioned are of the normal form. If the planula fails to attach itself when it comes to settle down, it is shaped like a disc and both the tentacles and skeletons come to appear, rotating ceaselessly on a bottom of vessel. Such an abnormal form has already been observed on *Agaricia fragilis* by MAYOR ('15) and *Pocillopora bulbosa* by STEPHENSON ('31). On the other hand it was reported by EDMONDSON ('29) that the tentacles of larvae of *Pocillopora damicornis* and *Cyphastrea ocellina* appeared during their swimming stages.

Another deformity of planula is occasionally found, that is, two planulae are fused together with their aboral extremities, while the other ones are free. Consequently the planula is shaped like a fork, but swims actively like the normal one. The same kind of deformity was found in *Siderastrea radians* (DUERDEN, '04) and also in *Acrohelia horrescens* (KAWAGUTI, '41).

(3) THE INTERNAL STRUCTURE.

As the fixing reagent several kinds of solution were attempted, but in all cases the tissues of planulae considerably shrank. I used to employ Bouin's solution by which comparatively good specimens were obtained. The fixed materials were imbedded into a paraffin, cut into serial sections of 5 μ to 10 μ in thickness, stained with Heidenhain's iron haematoxylin and orange G and also Delafield's haematoxylin and eosin.

The internal structure of the present larva, as is illustrated in Text-fig. 2, rather resembles that of *Siderastrea* larva which DUERDEN ('02, '04) observed in full details. In the ectodermal tissue two kinds of gland cells are found. The larger one is present in a great quantity and some fine granules which are not affected by the haematoxylin are contained in it. Another slender gland cell is situated more or less abundantly between the larger ones and is filled with granules which are well stained by the haematoxylin. The nematocysts are very few and are of much larger size than the gland cells. The outer surface of ectoderm is ciliated.

The ectoderm of the aboral region is thickened in particular, especially in the aboral extremity, forming a special layer. It consists of nerve fibers, supporting cells and numerous slender gland cells, while only a few of nematocysts are found. The stomodaeum is developed to some degree and is opened to the exterior through the oral aperture.

According to DUERDEN ('04), in the ectodermal thickening of *Agaricia agaricites* the fine nerve fibers extend horizontally from the mesoderm and besides a few of the gland cells and the nematocysts are found. The latter is present more or less abundantly. In *Isophyllia dipsacea* such nervous layer as above mentioned is strongly developed and in *Siderastrea radians* it represents the nervous elements consisting of sensory cells, supporting cells and a few of gland cells.

The gastro-coelomic cavity is not yet differentiated and the interior of body is occupied by a loose vacuolated tissue. The outer layer of endodermal tissue becomes somewhat compact, in which a great number of large and spherical Zooxanthellae are found. These symbiotic algae are aggregated in particular around the oral aperture.

The mesenteries are strikingly developed even in such a free swimming stage. Those of six pairs are arranged bilaterally about the central plane including the longitudinal axis of oral aperture. Of the mesenteries four pairs reach the stomodaeum and unite to it, representing the complete mesenteries, while the other two pairs are set free from the stomodaeum, forming the incomplete mesenteries. The former is so-called Edwardsian mesenteries. The aspect of mesenterial arrangement will be illustrated in Text-fig. 2 in which the Roman numerals denote the following mesenteric pairs:

I. The dorsal moiety of the ventro-lateral pair.

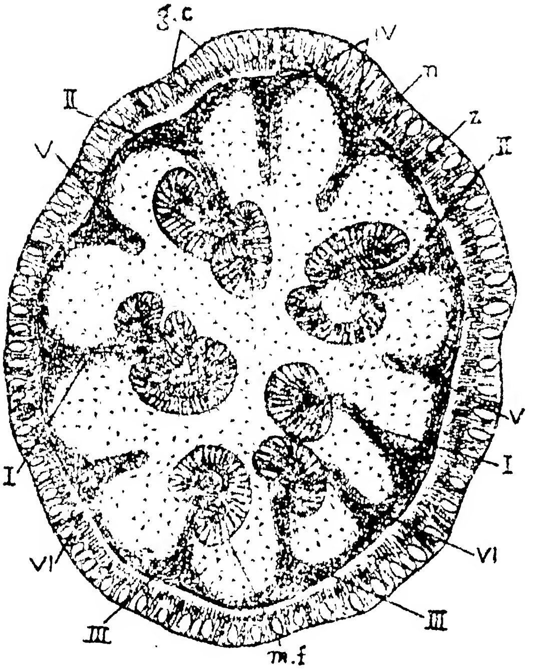
II. The dorsal moiety of the dorso-lateral pair.

III. The ventral, posterior or sulcar directives.

IV. The dorsal, anterior or sulcular directives.

V. The ventral moiety of the dorso-lateral pair.

VI. The ventral moiety of the ventro-lateral pair.



Text-fig. 2. Showing the internal structure of planula of *Pocillopora damicornis caespitosa* (DANA). The figure is taken from the transverse section, 10 μ thick, stained with Heidenhain's iron haematoxylin and orange G. × *ca.* 110. I–VI, mesenteries; g. c, two kinds of gland cells; m. f, mesenterial filament; n, nematocyst; z, Zooxanthella.

Of all the mesenteries the pairs of directives alone are isocnemic and all of the remaining pairs are anisocnemic (DUERDEN, '02). All of them bear the mesenterial filaments and those of Edwardsian are more strongly developed. In the filaments a considerable number of gland cells, nematocysts and Zooxanthellae are found (Text-fig. 2).

(4) THE MOVEMENT

The planula swims actively with its aboral extremity directed anteriorly. When kept in a glass vessel, it swims rapidly near the surface of water or descends to the bottom, creaping there. Sometimes it applies its oral or aboral extremity to the bottom and rotates its body like a top. Soon after it leaves the bottom, ascends to the surface, turning into spherical or hemispherical in shape and floats on it.

The movement of planula is performed by rapid rotation of body about its longitudinal axis. When swims upward or downward, the planula describes some spiral course and when advances in horizontal direction, it takes some wavy course due to a slight inclination of body axis. The direction of spiral is left handed or counter clockwise in the downward movement and is right handed or clockwise in the upward movement when observed from the aboral side of planula. Such a spiral movement found in the upward movement was also observed on that of *Favia fragum* (DUERDEN, '02), *Pocillopora bulbosa* (STEPHENSON, '31) and *Fungia actiniformis* (ABE, '37).

Then the velocity of the movement was measured. Many planulae spawned on the day were kept in a square glass vessel, 14 X 7 X 7 cm., in which two parallel lines were drawn on each broader wall. The distance of two lines is 10.0 cm. and the upper lines is situated at 12.5 cm. in height from the bottom. It was, then, measured how it took to pass through this distance in both the up- and downward movement and the results were as follows (Table 3):

TABLE 3. Showing the time required to pass through the distance of 10 cm. in the up- and downward movement of planulae of *Pocillopora damicornis cæspitosa* (DANA). (water temperature: 29.0° C.)

Animal No.	Upward movement in sec.	Downward movement in sec.
1	22.2	15.4
2	22.6	16.6
3	29.4	17.2
4	31.0	17.4
5	32.2	17.6
6	32.4	18.6
7	32.6	18.8
8	33.0	19.0
9	33.6	19.2
10	33.8	19.6
11	35.0	20.0
12	35.0	20.0
13	35.4	20.2
14	35.8	21.0
15	36.0	21.2
16	36.2	21.6
17	36.6	21.8
18	36.8	22.0
19	37.0	22.0
20	37.2	23.0
21	38.0	23.4
22	38.2	23.6
23	40.4	23.6
24	41.2	23.6
25	44.0	24.0
26	46.0	24.4
27	47.2	24.6
28	48.0	25.0
29	52.4	27.0
30	62.0	27.2
Average	37.4	21.3

The velocity of movement varies in proportion to the size of spiral. As a rule, however, the upward movement is acted more slowly than the downward one and when the rate of the movement is converted into the distance to be advanced by planulae for 30 seconds, it will be known that they can swim upwards at the rate of 80.6 mm. and also can swim downward at the rate of 140.8 mm. Thus the planulae of the present species move much more speedy than those of *Fungia* (ABE '37).

(5) THE FLOATING PERIOD AND SETTLEMENT.

At first the planulae swim actively and aggregate at a light side of the vessel, but when it comes to the end of the first day after extrusion, they become less active wandering near the bottom. If they are kept in a vessel in which a light and dark sides are strictly distinguishable, it will be noticed that they show positive phototropism in the early stage of extrusion, while when it comes to the time to settle down, they show negative phototropism. Such behavior as given above was also found in *Fungia* larva (ABE, '37). On the other hand, according to KAWAGUTI ('41) who made studies on the tropisms of several species of coral planulae, all the planulae exhibited positive phototaxis to some degree in proportion to the intensity of light, and among them *Pocillopora* larvae responded to the strongest light.

The planulae usually settle on the bottom of vessel, but sometimes a few of them attach themselves to the wall just beneath the water level. The latter case, however, may be abnormal and it seemed to me that they were pressed against to the wall by the surface tention of water and then were sticked on it unnaturally during floating.

The free swimming or floating period has been investigated on several species of coral larvae from such a view point that it is the main controlling factor affecting the geographical distribution of corals, because they have to be transported by the tidal current during floating. As for the period of the present coral, six hundreds of planulae were examined and the following results were obtained (Table 4):

The table shows that about 92 % of planulae settled down and the remaining 8 % were failed to attach and died out. The planulae were floating for nine days, but most of them settled down within five days after extrusion, of which about 63 % were finished the settlement within two days. Furthermore a majority of planulae were attached themselves on the second day after extrusion.

According to EDMONDSON ('29), the floating period of larva of *Pocillopora cespitosa* is from three to eighteen days and that of *Cyphastrea ocellina* is from three days to one month. The larva of *Pocillopora bulbosa* attached themselves within twenty-four hours after extrusion, but occasionally it takes from two to three days or over a week (STEPHENSON, '31). ABE ('37) reported that the floating period of *Fungia* larva is from two to three days and that of *Goniastrea aspera* is from sixteen to twenty-three days.

TABLE 4. Showing the number of planulae of *Pocillopora damicornis cespitosa* (DANA) which settled down every day under the normal laboratory conditions and also in the total darkness.

Days after extrusion	Normal laboratory condition	Darkness
1	111	40
2	299	51
3	91	4
4	41	8
5	43	3
6	7	8
7	1	3
8	0	3
9	1	6
10		2
11		2
12		1
13		1
Total of settled planulae	549	132
Total of dead planulae	51	18
Rate of settlement	92.1%	88.0%

Then the behaviors of planulae kept in the total darkness were examined. Many planulae were kept in the rectangular glass vessel and were placed in the experimental dark room. They were observed in the morning, at noon and in the night every day, and illuminated from one side by 100 w. electric lamp at a distance of 30 cm. apart from the vessel when examined. Whenever examined, they were floating on the surface of water or swimming near it and in an instant affected by light sinking down rapidly and straightly to the bottom, not describing the spiral course. Soon after they began the normal movement, continuing it as far as they were illuminated.

It has already been noticed that the floating period was prolonged or the planulae could not settle down at all when they were kept in the darkness. Such phenomena as given above were recognized also in other shallow water corals as *Agaricia fragilis* (MAYOR, '15), *Cyphastrea* *ocellina* (EDMONDSON, '29) and *Fungia actiniformis palauensis* (ABE, '37), while the larvae of *Dendrophyllia manni*, one of the deep sea corals, were not affected by the absence of light (EDMONDSON, '29). As for the present species, only slight delay of floating period and also slight decrease of the rate of settlement in the darkness were observed.

(6) THE BEHAVIORS OF PLANULA IN THE DILUTED SEA WATER.

It may frequently happen that the coral larvae are exposed to the heavy rain of the tropics during floating on the shallow reef-flat or are transported to the mouth of some river by the surface current of sea water. In these cases they have to meet with sea water of low salinity. It will be significant to know the behaviors and power of resistance of planulae in such circumstance as above mentioned in relation to the geographical distribution of corals. In this viewpoint 400 of *Pocillopora* larvae were kept in sea water diluted with rain water and the following results were obtaind (Table 5):

TABLE 5. Showing the number of planulae of *Pocillopora damicornis cespitosa* (DANA) settled down every day in different dilutions of sea water.

Days after extrusion	Dilutions of sea water			
	50 %	60 %	80 %	90 %
1	84	108	132	153
2	13	32	10	26
3	23	9	24	39
4	3	1	11	39
5	0	0	12	15
6	0	2	2	8
7		0	0	4
8		0		2
10				1
13				0
17				2
Total of settled planulae	123	152	191	289
Total of dead planulae	277	248	209	111
Rate of settlement (%)	30.7	38.0	47.8	72.3

In Table 5, it will be noticed that the planulae were capable of attachment even in sea water diluted with equal volume of rain water (recorded as 50 % in the table), increasing the rate of settlement in proportion to the increase of salinity. A majority of planulae settled down on the first day after extrusion and it may be considered that it requires a short period to settle down in such an unfavourable environment.

2. *The postlarval development*

(1) THE REARING METHOD.

In order to make observations most easily on the development of planulae after attachment, some small rectangular vessels were prepared. Each of them was made with five of slide glasses (35 X 76 mm.) and its broader walls were inclined to some degree. The other two slides were leaned alternately against the walls. Many vessels, thus made, were arranged on the desk which was covered with black cloth and were placed near the window. Usually fourty of planulae were kept in each vessel, but if necessary, much more of them were taken in it. The vessel was filled with 70 cc. of sea water which were renewed once every day.

Wherever the planulae settle down, the vessel was readily divided into the slides to which the young polyps attach, then the slides were put into another large vessel in which the polyps were reared for a long time. In this way they could be observed under a microscope without any difficulty.

The rearing of the polyps was carried out in the laboratory. They were supplied clear sea water, not filtered, which was drawn from the water passage outside the reef flat. The water was renewed once every day. At the beginning of the rearing they were supplied with muddy water taken from slight outside of the mangroove swamp, but most of them died out within a few days after extrusion and some surviving polyps developed into an abnormal form.

As to the food, they did not receive any artificial bait, but it seemed to me that the polyps aquired sufficiently some animal planktons as their food materials from surrounding sea water in which these animals were always contained in a great quantity.

Under such circumstances as above mentioned the settled polyps were able to grow well at least over four months and during this period they produced prosperously new individuals by the asexual reproduction. In the course of rearing, however, it is very necessary for young polyps to get rid of fine silts These are suspended in water in a great quantity, accumulating on the polyps and exceedingly prevent their development. In addition to these, some green algae must be removed from them. These plants grow actively all over the slide, covering the polyps and become the cause of their death.

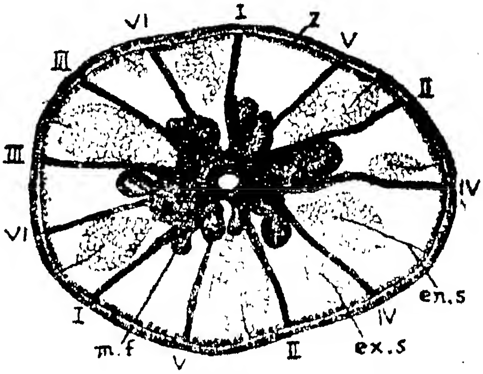
Furthermore, the polyps are often injured by another cause, that is, at first they settled firmly on the slide, but later on easily separated from it even with slight stirring of surrounding water, without any trace of destruction of the basal plate by which they adhere to the slide.. The same fact was ~~also~~ found in the other corals, *Seriatopora hystrix*, *Stylophora pistillata* and *Galaxea asper*, and it was thought that it was resulted from the invation of symbiotic algae into the basal plate. The algae under consideration has already been recognized by several authors as one of the destructive power of coral reef, and UCHIUMI ('42) has described on these data in his paper.

(2) THE DEVELOPMENTAL PROCESSES.

a. *The mesentery and tentacle.*

As soon as the planula settles down, it becomes flattened and is shaped like a disc. The newly-settled polyps show various developmental steps during twenty-four hours after attachment, and the diameter of disc ranges from 0.7 mm. to 1.4 mm. (Text-fig. 4, b-d'). The central part of disc is more or less swollen and the oral aperture is opened at its center. The margin of aperture is pale green in colouration having short cilia which are moved unceasingly. The cilia to be found on the body of free-swimming larva disappear shortly after attachment. The polyps are capable of expansion and contraction of body from the early stage and when undisturbed, extending their tentacles during daytime. They are strikingly sensitive to some mechanical stimulation, and even with a slight shock of surrounding water contract suddenly their soft parts, while in about two weeks after settlement they become less sensitive. On the contrary the *Fungia* larva (ABE, '37) begins to show the susceptibility to sound stimulus in about that stage.

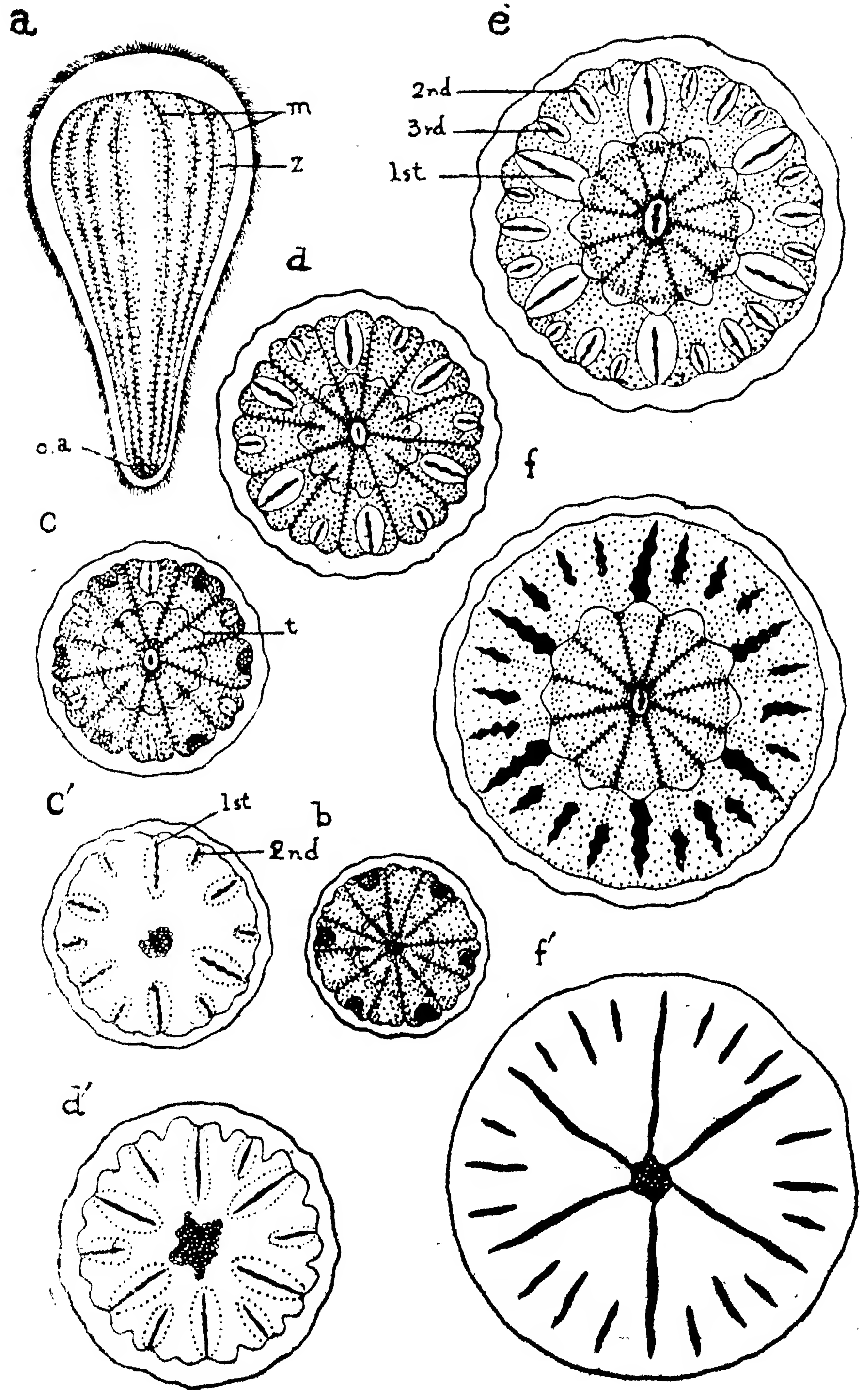
As already mentioned, six pairs of primary mesenteries or protocnemes (DUERDEN, '02) have already been developed in the free-swimming stage and the same aspect is maintained in the settled polyp. Now the gastro-coelomic cavities are differentiated and the entocoels and exocoels are arranged alternately (Text-fig. 3; 4, b-d). According to DUERDEN ('02), such an arrangement and an aspect of mesenteries represent the simplest form of Madreporaria.



Text-fig. 3. The transverse section of the newly-settled larva of *Pocillopora damicornis caespitosa* (DANA), 10 μ thick, cut through the stomodaeal region, stained with Heidenhain's iron haematoxylin and orange G. Showing the aspects of mesenteries and septa. × ca. 50.
I–VI, mesenteries; en. s, entosepta; ex. s, exosepta; m. f, mesenterial filament; z, Zooxanthella.

The tentacles appear in the strikingly early stage. Immediately after attachment, twelve of tentacles are given rise simultaneously on the wall of mesenteric chambers. They are visible as whitish swellings and are situated at about equal distance apart from the mouth. On the following day they become larger (Text-fig. 4, e) and on the fourth day their stems are more or less lengthened (Text-fig. 5, a). On the fifth day the tentacles are further elongated being shaped like a rod. Their tips are spherical, presenting white colouration being the devoid of Zooxanthellae and are armed with numerous nematocysts, while the algae are distributed rather abundantly in the stems. In this stage it will be often found that the polyp catches some Copepoda of rather large size and swallows it into its stomodaeum.

Thus the primary or prototentacles each consisting of six of ento- and exotentacles appeared in such an early developmental step. These two kinds of tentacles are arranged alternately at about equal distance apart from the mouth, forming a simple tentacular crown. Such an aspect of tentacles has been maintained for a long time without any


a
m
z
o.a
e
2nd
3rd
1st
d
c
t
c'
1st
2nd
b
f
f'
d'

Text-fig. 4.

Showing the development of the larva of *Pocillopora damicornis cæspitosa* (DANA).

a planula. m, mesenteries; o. a, oral aperture; z, Zooxanthella. × ca. 30.

b young polyp, just after attachment, observed from oral side.

c, d young polyp, within one day after attachment, oral view. t, tentacle.

c', d' aboral view of c and d respectively. 1st, the first cycle of septa; 2nd, the second cycle of septa.

e young polyp, the second day after attachment. oral view. 1st and 2nd, same as above; 3rd, the third cycle of septa.

f young polyp, the third day of attachment. oral view; f' aboral view of f.

b–f', × ca. 19.

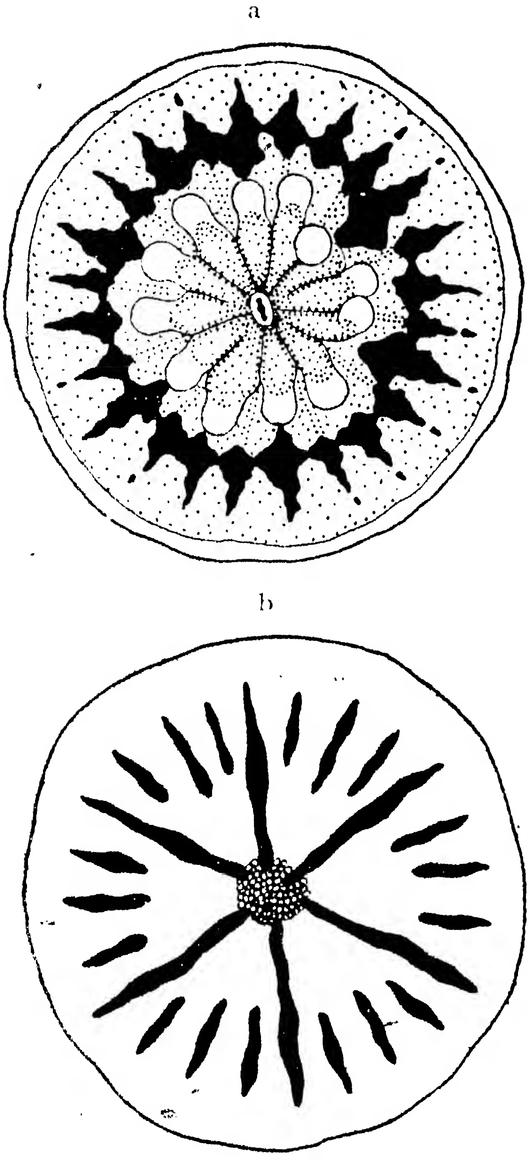
noticeable change except their further elongation. As abnormal cases, however, four, six, nine or eighteen of tentacles were found in some polyps.

b. The skeleton.

As soon as the planula settled down, the basal plate is secreted at the base. It is thin and irregular calcareous layer, distending somewhat farther than the soft parts and looks projecting to some degree outside the tissues. Soon after the attachment of the planula the septa appear also. These are the first cycle or entosepta arising from the basal plate in each entocoel. Soon later or nearly at the same time the other six of septa appear at the base of each exocoel, forming the second cycle or exosepta. These two cycles of twelve septa are visible from the exterior through the walls of mesenteric chambers as small irregular corpuscles which as yet are not united to form definite ridges. Each of them is arranged bilaterally or radially in the basal plate (Text-fig. 4, c-d).

On the second day of attachment each septum is shaped like a needle having rugged periphery, and in addition to these septa, the third cycle of twelve septa appear between them. As the result, twenty-four of septa have appeared and are arranged radially or bilaterally at the base of the polyp (Text-fig. 4, e).

On the third or fourth day of attachment the septa become rod-like and those of the first cycle alone grow much longer than the others, some of them reaching to the central upgrowth of the basal plate.



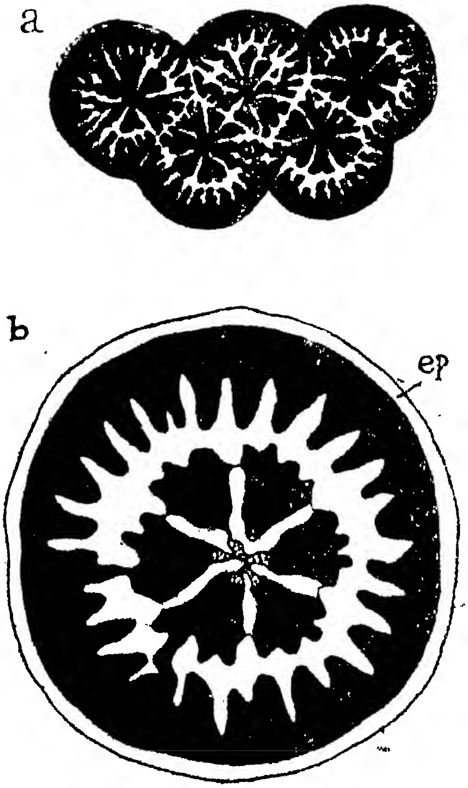
Text-fig. 5. Showing the development of tentacles and septa in young polyp, the fourth day of attachment, in *Pocillopora damicornis caespitosa* (DANA). × ca. 19. a, oral view; b, aboral view.

The exosepta are slightly longer than the remaining ones. All of them increase the height and project upwards irregularly (Text-fig. 4; f, f'; Text-fig. 5; a, b).

In the following developmental step the ridges of septa are enlarged and come to contact with each other. Soon later they are fused together and thus the theca is formed, and at the same time the calyx or calycle is established (Text-fig. 6, b).

The basal parts of endosepta are enlarged toward the center of calyx and meet with the central upgrowth of basal plate, then are fused together. The fusion takes place between some septa or all of them and in all cases it is followed with the bilateral symmetry. The region formed by the fusion steeply projects upwards and thus a skeletal protuberance or columella is resulted at the center of basal plate. Consequently the columella is built on the upgrowth of the plate. Such a structure as given above is usually developed within five days after attachment. At about this stage the epitheca appears also in some polyps (Text-fig. 6, b), while in the other polyps it does not occur even in the later stage.

Meanwhile, when two or more of planulae settle on the slide in the close neighbourhood of others, they are easily fused and thus the aggregated colonies are formed. As a rule each individual of the colony has common theca, but in some cases each polyp is strictly partitioned by the skeletal boundaries (Text-fig. 6, a). Such a colony formation has already been noticed in *Siderastrea radians* (DUERDEN, '04), *Maeandra areolata* (BOSCHMA, '29), *Pocillopora bulbosa* (STEPHENSON, '31) and *Fungia actiniformis* (ABE, '37).



Text-fig. 6. Showing the skeletons of young corals of *Pocillopora damicornis caespitosa* (DANA).

a, Skeleton of aggregated colonies, the third day of attachment. Oral view, × ca. 11.

b, Skeleton of young polyp, the fourth day of attachment. Oral view, ep, epitheca. × ca. 34.

After the establishment of theca the skeletons of polyp mainly grow upwards and the theca becomes conical, from which many processes being projected. The asexual reproduction or budding takes place actively and the skeleton of daughter polyp is formed at the lower part of the theca. One of the processes of theca becomes the columella of bud and its adjacent skeletons are transformed into the septa of bud, consequently the secondary modification of skeleton of parent polyp is given rise following the increase and growth of bud. After this the skeleton increases its weight and becomes much more rigid and complicated day by day, but the number and arrangement of septa are maintained without any essential change at least for four observable months.

Thus the fundamental structures of mesenteries, tentacles and skeletons have already been developed within about a week after attachment, and in the following stages the asexual reproduction is mainly performed. From these facts the developmental steps of the present coral may be distinguishable into two stages, the earlier and the later.

The aspect of the protocnemes, the rapid appearance of both the prototentacles and of the twenty-four of septa in such an early stage, the simultaneous or nearly simultaneous occurrence of ento- and exotentacles and also of ento- and exosepta, and besides the form and arrangement of all septa, have closely resemblance in all respects to those of *Pocillopora bulbosa* (STEPHENSON, '31) and it may be considered that these features are the characteristic of this genus.

SUMMARY

1. One of the reef-building corals, *Pocillopora damicornis cespitosa* (DANA) belongs to the Family *Seriatoporidae*, Suborder *Madreporaria Imperforata* (EGUCHI, '38). Some features and behaviors of the larva and its postlarval development are dealt with in the present paper.

2. The larvae or planulae are liberated every month throughout the year in accordance with the lunar periodicity, namely, the planulation begins to occur several days before the time of the new moon and reaches its maximum at or about the time of the new moon and it lasts for several days. For some ten days including the time of the full moon the planulation does not take place.

3. The planula is brown in hue due to the symbiotic Zooxanthellae

to be found in its endoderm in a greart quantity. The mesenteries are visible as longitudinal striations through its ciliated surface. The oral aperture opens at one extremity of the body.

4. In the ectoderm two kinds of gland cells are present. The ectoderm of the aboral extremity of body is thickened in particular forming the nervous layer. It consists of the nerve fibers, supporting cells and besides a great number of gland cells. Only a few of nematocysts are found.

5. The six bilateral pairs of mesenteries have already been developed in the stage of free-swimming planula. Of these pairs four are complete and are united with the stomodaeum, while the remaining two are incomplete and are free from the same. All of them bear the mesenterial filaments. The gastro-coelomic cavity is not yet differentiated.

6. The planula swims actively with its aboral end directed anteriorly. Its movement is performed in rapid right-handed rotation around the longitudinal axis of the body. In addition to this, it takes the wavy course when advances horizontally and also the spiral course when advances upwards and downwards. It can swin at the rate of about 80 mm./30 sec. when ascends and about 140 mm./30 sec. when descends in the aquarium.

7. At first the planula shows positive phototropism, while it turns into negative when the time of settlement is approached. The floating period of the planulae lasts from one to nine days and most of them will settle down within five days after extrution. Moreover a majority of settlement is accomplished on the second day.

8. Even in the total absence of light the planula is capable of settlement, but the floating period is more or less prolonged. Under the same conditions as above most of the planulae settle down within two days after extrusion and the rate of the settlement is slightly decreased than that of the normal case in the laboratory.

9. In the diluted sea water the planula is also able to settle down and even in 50 % sea water about 30 % of the planulae can settle.

Most of them do so within one day after extrution.

10. The settled larvae can be reared for a long time in the laboratory. At first these young corals are very sensitive to some mechanical stimulus, while after about two weeks they become less susceptible.

11. In the newly-settled larva six pairs of mesenteries have already been developed and both six endo- and exocoelic chambers were established. Soon after both six endo- and exocoelic tentacles are given rise from respective walls of endo- and exocoels. The Zooxanthellae are richly distributed all over the soft part of the polyp.

12. All the tentacles appear simultaneously and the endo- and exocoelic ones are arranged alternately on the same level, forming a single tentacular crown. The features of the mesenteries are nearly similar to those of the swimming larva.

13. The skeleton is also developed at a very early stage. On the first day after attachment the cycle of six entosepta and the cycle of six exosepta are developed from the basal plate. The septa of these two cycles are arranged alternately. On the second day the third cycle of twelve septa occurs between the first and second cycles of septa. As the result twenty-four of septa are arranged radially or bilaterally. Among these the endosepta alone are prominent in length, and their basal parts reach the central thickening of the basal plate.

14. On about the fifth day after attachment the upper parts of all the septa spread and are fused together in their enlarged region. Thus the theca is constructed and at the same time the calyx is established. At this stage the columella is also developed and the first budding begins to occur. In some polyps the epithecal structure is developed.

Thus the fundamental differentiation of the mesenteries, tentacles, septa and the other skeletal structures have already been established within only five days after attachment.

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THE LARVA AND POSTLARVAL DEVELOPMENT OF SOME REEF-BUILDING CORALS II. *STYLOPHORA PISTILLATA* (ESPER)

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(With two Text-figures and one plate)

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INTRODUCTION

While enjoying the long stay in the Palao Islands of the former mandated South Sea Islands of Japan, I had an opportunity to obtain several kinds of coral larvae, and could observe their behaviors and also was able to trace their developmental sequence. Among these larvae, I had already reported on *Pocillopora damicornis cespitosa* (DANA) ('46). *Stylophora pistillata* (ESPER), which is one of the reef-building corals, belongs to the same family as *Pocillopora*, viz., Seriatoporidae, but the colonial forms of these two species are very different.

It is not an easy task to obtain the larvae of the present coral, but the parent colony is alive much longer time than that of *Pocillopora*, and its settled larvae can grow well extending a long time under the laboratory conditions. Thus the *Stylophora* larva proved to be one of the most excellent materials for the studies of the coral development as well as to observe the features of larva. Furthermore, when the developmental steps of the two species of corals of Seriatoporidae are compared with each other, it may throw some light upon the mechanism by which the two kinds of coral colonies of different shapes are formed.

The present work was carried out at the Palao Tropical Biological Station. Before going further the author wishes to acknowledge his indebtedness to the Japan Society for the Promotion of Scientific

Research for its financial aid. His hearty thanks are also due to Emer. Prof. SHINKISHI HATAI, the former director of the station given above, for his kind guidance during the investigation. Further he wishes to express his sincere thanks to Prof. Dr. SANJI HÔZAWA of the Tôhoku Imperial University for the cordial guidance given to the present writer after he left the station.

MATERIAL

Stylophora pistillata (ESPER) is one of the branching corals consisting of numerous minute corallites. It is commonly found in the fringing reef of Iwayama Bay of the Korror Island in the Palao group, but it does not aggregate at one habitat, so it is not so easy to collect many materials in a short time. Each branch of the colony is big and short with blunt tips and is shaped like a finger of man. The skeleton of the colony is dense and rigid and is very heavy. The coloration of living coral is dark brown or brownish yellow and as a rule the tips of each branchlet are pale. The soft part of the polyp is expanded during the night only, as are found in many other corals, both in the natural habitat and in the laboratory.

All of the observations on the larva and its postlarval development were made in the laboratory. The method of collection of materials and the same of rearing of both larvae and settled polyps, and also the laboratory conditions were all the same as in the case of *Pocillopora* (ATODA, '46).

OBSERVATION

1. *The larva or planula*

I. THE PERIOD OF EXTRUSION.

The extrusion of the planula was examined using several number of colonies at one time of the observation. Each of them was about 400 grams in weight and was collected from different habitats. Although the corals were alive for a considerably long period in an aquarium, but the observations were made for about three days of each colony and then the other fresh materials were successively brought to examine. As is shown in Table 1, the observations were carried on for twenty-nine months extending from 1938 to 1941 and during this period the planulation were found in the following months:

1938··Jul., Aug., Oct., Nov. 1939··Jan.—Apr., Jun., Aug., Sep.
1940··Jul., Oct., Dec. 1941··Mar.—Jun.

From these data it can be known that the planulation occurs every month throughout the year as in the case of *Pocillopora* (ATODA, '47).

TABLE 1. Showing the data of the extrusion of planula of *Stylophora pistillata* (ESPER) during the period extending from 1938 to 1941.

Date of collection	Moon's age	Planulae extruded (+), or not extruded (—)	Date of collection	Moon's age	Planulae extruded (+), or not extruded (—)
1938					
Jun. 1	4	—	23	30	—
15	18	—	25	2	—
16	19	—	26	3	—
17	20	—	28	5	—
25	28	—	29	6	—
26	29	—	30	7	—
28	1	—	Oct. 18	25	—
Jul. 21	24	+	21	28	—
22	25	+	22	29	—
23	26	+	24	2	—
Aug. 19	24	+	25	3	—
20	25	+	Nov. 15	24	+
21	26	+	16	25	+
25	1	—	18	27	+
26	2	—	19	28	—
Sep. 3	10	—	22	1	—
4	11	—	23	2	—
11	18	—	29	8	—
12	19	—	Dec. 5	14	—
13	20	—	6	15	—
22	29	—	11	20	—

	12	21	—	Apr.	2	13	—
	15	24	—		3	14	—
	21	30	—		7	18	—
	22	1	—		8	19	+
1939				May.	4	15	—
Jan	4	14	—		5	16	—
	5	15	+		10	21	—
	8	18	—	Jun.	10	23	+
	9	19	—		15	28	—
	15	25	—		16	29	—
	16	26	—		21	5	—
	18	28	—		23	7	—
	19	29	—		24	8	—
	21	2			27	11	—
	22	3			28	12	—
	24	5	—	Jul.	4	18	—
	25	6	—		5	19	—
	30	11	—		15	29	—
	31	12	—		16	30	—
Feb.	2	14	—		19	3	—
	3	15	—		21	5	—
	4	16	—		22	6	—
	7	19	—		23	7	—
	10	22	+		27	10	—
	11	23	+		28	11	—
	14	26	+		31	15	—
	16	28	+	Aug.	1	16	+
	20	2	—		4	19	+
	21	3	—		12	27	—
	24	6	—		15	1	—
	25	7	—		18	4	—
	26	8	—		19	5	—
	28	10	—		25	11	—
Mar.	1	11	—		26	2	—
	2	12	—	Sep.	1	18	—
	7	17	—		5	22	+
	8	18	—		7	24	+
	12	22	+		9	26	—
	14	24	+		10	27	—
	18	28	—		14	2	—
	23	3	—		15	3	—
	25	5	—		16	4	—

19	7	—	Oct. 2	2	—
20	8	—	3	3	—
25	13	—	22	22	+
26	14	—	23	23	—
Oct. 3	21	—	Nov. 26	27	—
4	22	—	Dec. 19	21	+
7	25	—	21	23	—
11	29	—	24	26	—
1940			1941		
Jun. 27	22	—	Mar. 20	23	+
Jul. 4	29	—	21	24	+
5	1	—	Apr. 18	20	+
6	2	—	May. 9	13	—
30	26	+	10	14	—
Aug. 1	28	—	12	16	—
2	29	—	13	17	—
6	3	—	16	20	+
7	4	—	21	25	—
8	5	—	24	28	—
9	6	—	25	29	—
24	21	+	Jun. 14	20	+
28	25	+	16	22	+
Sep. 28	27	+	17	23	+

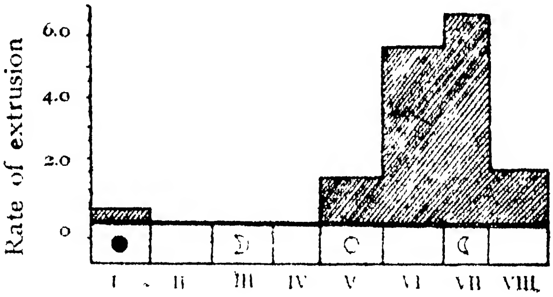
Then the relation between the planulation and the phases of the moon was examined by the same way as used in the case of *Pocillopora* (ATODA, '46). That is to say, all the data concerning the planula extrusion were summarized according to the moon's age only and the following results were obtained (Table 2, Text-fig. 1):

From the data given above, it may be concluded that the planulation begins to occur at about or just at the time of the full moon, and thereafter it becomes active. When it comes to the time of the last quarter or thereabout, the extrusion takes place most frequently. After this phase it rapidly decreases and is finished at about the time of the new moon. For about ten days before the full moon none of the planulae is produced. Thus the planulae of *Stylophora pistillata* (ESPER) are spawned every month throughout the year in accordance with the lunar periodicity.

TABLE 2. Showing the relation between the planulation and the moon's age in the case of *Stylophora pistillata* (ESPER).

Division	Moon's age	Planulae extruded	Planulae not extruded	No. of time of observation	Rate of extrusion
I	28 – 1	1	27	28	0.04
II	2 – 5	0	17	17	0
III	6 – 9	0	12	12	0
IV	10 – 12	0	17	17	0
V	13 – 16	3	18	21	0.14
VI	17 – 20	10	8	18	0.56
VII	21 – 23	19	9	28	0.68
VIII	24 – 27	4	23	27	0.17

The other examples of the planula liberation controlled by the phase of the moon have already been noticed in the cases of *Pocillopora bulbosa* (MARSHALL and STEPHENSON, '33), *Maeandra (Manicina) areolata* (YONGE, '35), *Fungia actiniformis palauensis* (ABE, '37) and *Pocillopora damicornis caespitosa* (ATODA, '46), and thus this time another characteristic type of the periodicity was newly added by the present coral.



Text-fig. 1. Showing the planulation of *Stylophora pistillata* (ESPER) taking place in relation to the phases of the moon.

2. THE GENERAL FEATURES.

The planula just extruded is usually slender and is shaped like a short rod. Shortly after it turns into a pear shape and its narrower end becomes an oral extremity, but in some polyps the broader one becomes the oral. It is smaller than the *Pocillopora* larva in size, and when measured of the elongated one, the longitudinal axis of body is about 1.1 mm., and the broadest width of the transverse axis is about 0.3 mm. It is brown in colour due to the symbiotic Zooxanthellae to be found abundantly in the body, while the aboral extremity is whitish being devoid of the algae. The body surface is covered with short cilia and the mesenteries are visible as several dark longitudinal striations through the transparent body wall (Text-fig. 2, A).

As it is shown in Plate I, 1, the ectoderm is well-developed and is of considerable thickness. There are two kinds of gland cells in it and both of these cells are distributed in a nearly equal quantity, and as a rule they are found more abundantly in the upper part of body. These cells are always filled with fine granules. The ectoderm forming the aboral region of body, especially in the aboral extremity, is thickened in particular, exhibiting nearly the same aspect as shown in the case of *Pocillopora* (ATODA, '46). This special layer consists of the nerve fibers, supporting cells, numerous slender gland cells and of a few of nematocysts, and may correspond to the ectodermal nervous layer observed by DUERDEN ('02, '04) in *Agaricia agaricites*, *Isophyllia dipsacea* and also in *Siderastrea radians*.

The oral aperture has already been opened and the stomodaeum is more or less differentiated.

The interior of larva is occupied by a vacuolated tissue. The gastro-coelomic cavity is not yet differentiated, but a narrow space found just beneath the stomodaeum will become this cavity in future. The outer layer of endoderm is somewhat compact, bearing some number of large and spherical Zooxanthellae arranged in a row. This kind of algae is most abundant in the endoderm surrounding the oral aperture, but never found beyond the endoderm. In *Isophyllia dipsacea*, *Siderastrea radians* (DUERDEN, '02, '04) and also in *Euphyllia glabrescens* (KAWAGUTI, '41) some of these algae are discovered also from the ectoderm.

In the free-swimming larva of *Pocillopora* (ATODA, '46) six pairs of protocnemes have already been developed, while in the present species only three pairs of mesenteries are found. These are arranged bilaterally and are united with the stomodaeum in their inner margins forming the complete mesenteries. All of them bear the mesenterial filaments.

3. THE FLOATING PERIOD AND SETTLEMENT.

As soon as the planula is set free from its parent, it swims actively towards every direction in the aquarium. Usually it advances with the broader and rounded aboral extremity directed anteriorly, but occasionally the narrower extremity becomes anterior. In all cases, however, the oral aperture is situated in the posterior of body when advances and it does not show any proof of reversal ciliary movement. It swims horizontally, taking up- and downward direction by the same way as observed on the *Pocillopora* larva (ATODA, '46).

At about the end of the first day of extrusion, the planula is more or less flattened and becomes like a sphere. It begins to move less actively than ever and will settle down before long. Most of the planulae settle on the bottom of aquarium, especially aggregating in the corner of bottom and walls. When the floating period was observed of 383 of planulae, the following results were obtained (Table 3):

TABLE 3. Showing the number of planulae of *Stylophora pistillata* (ESPER) settled down every day in the aquarium.

Days after extrusion	1	2	3	4	5	6	Total of settled larvae	% of settled larvae
No. of settled larvae	142	60	19	4	1	1	227	59.2

The table shows that the floating period of the planulae of the present species was from one to six days and during this period about 60 % of the planulae finished their settlement and the remaining 40 % failed to settle down. The planulae failed to settle continue to rotate on the bottom and will die out within about ten days. Most of the larvae finished the settlment within three days after extursion and among them the majority accomplished the same on the first day of extrusion. Thus the rate of settlement of the present coral is much poorer than that of *Pocillopora* (ATODA, '46), but the settlement is finished more quickly.

II. *The postlarval development.*

The larvae settled on the slide glass were easily obtained by the same method as was reported on *Pocillopora* (ATODA, '47). These young polyps have grown well for a long time in the laboratory.

As soon as the planula settled down, it turns flattened and becomes like a thin disc. It grows rapidly within one day after the attachment and the diameter of base of the settled larva is increased from about 0.7 mm. to about 1.2 mm. The central part of polyp body is slightly swollen and the mouth is opened there in center. It is greenish in hue and its inner margin is covered with cilia moving ceaselessly. The cilia found on the body surface of free-swimming larva disappear shortly after the attachment. The Zooxanthellae are freely distributed nearly all over the soft part, especially they are found most abundantly around the mouth (Text-fig. 2; B, C).

(1) THE MESENTERY AND TENTACLE.

As above mentioned only three pairs of complete mesenteries are found in the free-swimming larva, while shortly after the attachment six pairs of mesenteries have already been developed. These consist of four pairs of *Edwardsia* mesenteries and two pairs of incomplete ones. All of them are arranged bilaterally. The mesenterial filaments are developed along the inner margins of all mesenteries. The gastro-coelomic cavity is now differentiated and each six of ento- and exocoels are arranged alternately (Text-fig. 2, B).

The development of the tentacles begins at a very early stage. Shortly after the attachment, the tentacles are not yet discernible (Text-fig. 2, B), but on the latter half of the first day of attachment twelve of tentacles arise from the walls of both ento- and exocoels. They appear simultaneously as small white swellings and are situated on the central thickened part of polyp at about equal distance apart from the mouth (Text-fig. 2, C). After this the tentacles will become longer day by day (Text-fig. 2; H, I,), and on the fifth day of attachment their stems are considerably lengthened, looking rod-like in shape. The tips of tentacles are spherical and white in colour due to the absence of Zooxanthella. These tips are armed by a great number of nematocysts. From about this stage the polyp begins to catch actively its food by using the tentacles, and when it swallows some large animal as Copepoda, the mouth is strikingly enlarged. Within a week or nearly so the tentacles become much longer.

These twelve of prototentacles including both six of ento- and exotentacles are arranged ring-like around the mouth, thus forming a single tentacular crown. Of these the exocoelic ones are more or less longer than the others and this aspect will be maintained for a long time.

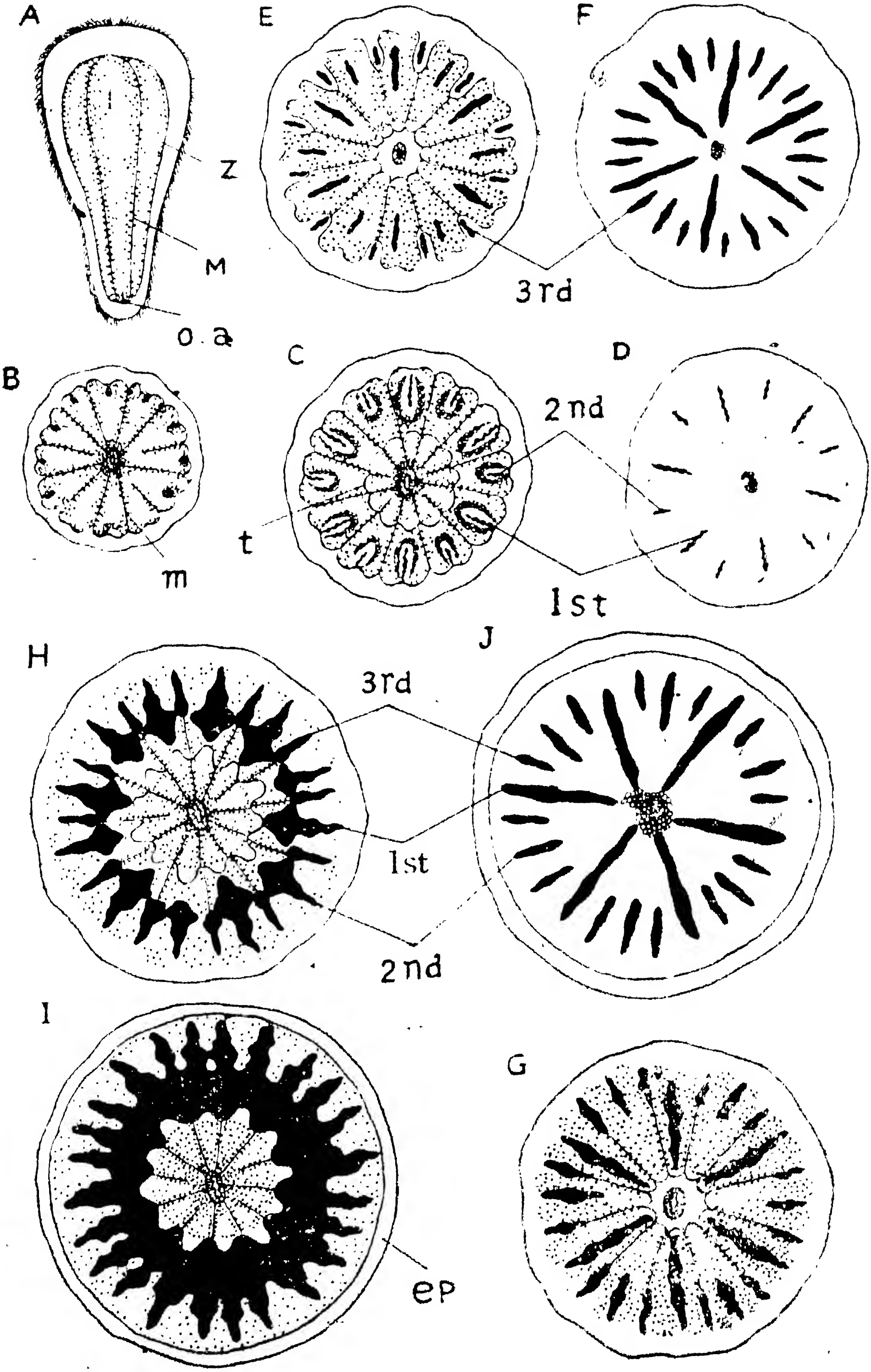
(2) THE SKELETON.

As soon as the planula settled on the slide, the basal plate is secreted at the base of the polyp. It is formed of a thin irregular calcareous layer and seems to project somewhat outside the soft part. The septa are not yet distinct, but some skeletal substances are visible in the base of each mesenterial chamber (Text-fig. 2, B).

On the latter half of the first day of attachment the six of septa appear as small irregular corpuscles in the basal plate of endocoels. These are the first cycle or order of septa or endosepta. Soon later or nearly at the same time another cycle or order of six septa or exosepta arises from the base of the exocoels and presents the same aspect as the endosepta (Text-fig. 2; C, D).

On the second day of attachment these two cycles of septa become needle-like and in addition to them the third cycle or order of twelve septa appears simultaneously among them. All of the twenty-four of septa arise from the basal plate as straight rod-like upgrowths and are arranged either radially or bilaterally (Text-fig. 2; E, F).

After this all of the septa are sharply projected upwards (Text-fig. 2, G). The upper ridges of both endo- and exosepta begin to enlarge (Text-fig. 2, H) and then they are fused together. In addition to them each dorsal tip of the third cycle of septa is also fused with them. As


A
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Text-fig. 2

Showing some developmental stages of the larva of *Stylophora pistillata* (ESPER). A—The planula, shortly after the extrusion. × ca. 40. o. a, oral aperture; m, mesentery; z, Zooxanthella. B—The larva, just after the attachment. Oral surface. m, mesentery. C—The polyp, in the latter half of the first day of attachment. The tentacles and septa appeared. 1st, the first cycle of endosepta; 2nd, the second cycle of exosepta; t, tentacle. D—Aboral surface of C. E—The polyp, in the beginning of the second day of attachment. The tentacles are contracted and twenty-four of septa appear. 3rd, the third cycle of septa. F—Aboral surface of E. G—The polyp, in the latter half of the second day of attachment, with contracted tentacles. H, I—The polyps, the third and fourth day of attachment, showing the development of tentacles and skeletons. 1st, 2nd, 3rd, each cycle of septa respectively; ep, epitheca. J—Aboral surface of I. B—J, × ca. 17.

the result the theca is formed and at the same time the calyx makes its appearance (Text-fig. 2, I; Pl. I, 2). Such developmental steps as given above are usually found on about the fourth day of attachment.

Of all the septa endocoelic ones are much more strongly developed than those of the second and third cycles, their inner extremity extending as far as the center of the calyx (Pl. I, 2). Then the inner edges of the endosepta come into contact and thus are united with each other. The union takes place between all septa or some of them, but always presenting the bilateral arrangement. The skeleton formed by the union sharply projects upwards and some spinous projections from the inner extremities of some endosepta are also fused with it and thus form the columella (Pl. I; 2, 3, 5). Such features usually appear within six or seven days after the attachment.

Hitherto, as to the columella formation, three different origins have been noticed. In the first place, at first the upgrowth arises from the center of the basal plate and some spinous projections of the inner extremity of both endo- and exosepta are fused together with it Then a secondary deposit of the calcareous matter is added to them and results the columella. Such a columella is seen in the cases of *Siderastrea radians* (DUERDEN, '04) and *Maeandra* (*Manicina*) *areolata* (BOSCHMA, '29). In the second place, from the beginning the columella appears as a separate projection from the basal plate, thus it has not any direct relation to the septa. This type of columella is found in *Galaxea asper* and *Acropora brüggemanni*. In the third place, the columella is formed only by the union of the inner extremities of endosepta. In this case the central upgrowth of the basal plate does not take part in the columella formation. The columellae originated from the former two means are the true ones and the latter is the pseudocolumella.

As above mentioned, although the columellae of the present coral, as well as those of *Pocillopora* (ATODA, '47), are developed on the central upgrowth of the basal plate, but they are mainly formed by the union of the inner extremities of endosepta and the upgrowth does not become their important constituents. Therefore, they resembles the pseudocolumella rather than the true one or it may be regarded as an intermediate form between these two kinds of columellae.

In this stage the peripheries of the basal plates of some polyps become thickened in particular, representing the origin of the epithecal structure, while in the other polyps it does not appear even in the later stage. However, when the polyp is surrounded by fine silts or dust, and also when some minute algae grow thickly around it, the margin of the plate is apt to be thickened (Text-fig. 2, I; Pl. I, 5).

In the subsequent developmental stages the skeletal structure becomes much more thickened and rigid, increasing its weight day by day and the thecal wall grows mainly upwards. In about eight days after the attachment the buds begin to be produced in the lower part of the theca and in accordance with the increase of buds and also with the development of their skeletons the secondary modification results in the skeleton of the parent polyp (Pl. I, 3, 5).

Now within about a week after the attachment the protocnemes, prototentacles, three cycles or orders of twenty-four of septa and the other structures have already appeared. After this these organs above mentioned are much more strongly developed, especially the skeletons present very complicated structure and such aspects as given above are maintained for a long period without any essential changes. In some cases, however, the polyp bears bifurcated tentacles, seeming to be abnormal and differing from the case observed in *Siderastrea radians* (DUERDEN, '04). In the latter case the entotentacles are always bifurcated. In other cases of abnormality there were some polyps lacking some of the septa (Pl. I, 5, a).

Thus the fundamental development of the mesenteries, tentacles and skeletons is accomplished within about a week or nearly so after the attachment, and in the following stages the asexual reproduction or budding chiefly takes place. The developmental processes of the coral *Stylophora pistillata* (ESPER) closely resemble in all respects those of *Pocillopora bulbosa* (STEPHENSON, '31) and of *Pocillopora damicornis cespitosa* (DANA) (ATODA, '47). On the other hand any conclusion concerning the mechanism, by which the different types of colonies are formed, could not be drawn in the present investigation, but it may be considered that such developmental sequences as above mentioned represent an essential features characterizing the family Seriatoporidae.

SUMMARY

1. *Stylophora pistillata* (ESPER) is one of the reef-building corals and belongs to the family Seriatoporidae. Some observations on the larva and the postlarval development of this kind of coral are dealt with in the present paper.

2. The larva or planula looks brown in colour due to the symbiotic Zooxanthellae which are to be found in the endoderm in a great quantity. The mesenteries are discernible as dark striations through the body wall. The oral aperture is opened at one extremity of the body.

3. In the ectoderm a considerable number of two kinds of mucus glands and a few of nematocysts are present. The ectoderm forming the aboral extremity of body is considerably thickened, comprising the nervous elements. The stomodaeum is more or less developed.

4. The internal cavity of the larva is occupied by the vacuolated

cells of the endoderm and the gastro-coelomic cavity is not yet differentiated. The cells forming the outer layer of the endoderm are arranged somewhat compact and the symbiotic algae are found among them. The algae never occur in the ectodermal tissue.

Three pairs of mesenteries are developed with their inner edges connected with the stomodaeum, viz., representing the complete mesenteries. All of them bear the mesenterial filaments along their inner margin.

5. The larvae are extruded every month throughout the year according to the phases of the moon. The planulation begins to occur at about the time of the full moon and reaches its maximum at about the time of the last phase of the moon. Then it is rapidly decreased and finishes at about the time of the new moon. For some ten days before the full moon, none of the planulae are expelled.

6. The floating period of the planulae lasts from one to six days. Most of them will settle down within three days after the extrusion and their majority do so on the first day of extrusion.

7. Shortly after the attachment, six pairs of the protocnemes will appear, of which four pairs are complete and the remaining two are incomplete. The gastro-coelomic cavity is now established. The traces of twelve prototentacles will appear simultaneously around the mouth on the respective walls of the mesenteric chambers. Of these tentacles the exotentacles are slightly longer than the endocoelic ones and these two kinds of tentacles are arranged alternately on the same ring, forming a single tentacular crown. The tips of all tentacles are devoid of Zooxanthellae, but they bear a great number of nematocysts. On about the fifth day of attachment the tentacles become active in catching some prey.

8. As soon as the larva settled down the basal plate is secreted at the base of the polyp. Within the first day of attachment the first and second cycles or orders of septa arise from the basal plate and on the second day the third cycle or order of twelve septa will appear

alternating with the former two kinds of septa. These twenty-four of septa are shaped like straight rods and are not bifurcated at their outer edges. All of the septa are arranged either bilaterally or radially and the principal septa (endosepta) of the first cycle are much more prominent in length than the others.

9. The outer edges of both of the first and second cycle of septa are enlarged laterally, and then are fused together. The tips of the third cycle of septa are also united with them, and thus the theca is constructed and at the same time the calyx is also established. Such developmental processes as given above are usually seen within four days after the attachment.

10. The columella is mainly formed by the fusion of the inner edges of endosepta and thus it is like the pseudocolumella in origin. The epitheca is also formed in some polyps, while in the other polyps it never appear for a long period. These structures are usually developed within about a week after the attachment.

11. The fundamental differentiation and development of the protocnemes, prototentacles, septa and of other structures are established within a week or nearly so, then the asexual reproduction will take place in the subsequent developmental stages. Consequently the secondary modification of the skeletal structures will be seen in accompany with the development and with the increase of buds. Thus the present coral has a strong resemblance in its postlarval developmental processes to another species of Seriatoporidae, *viz.*, *Pocillopora damicornis cespitosa* (DANA).

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EXPLANATION OF PLATE I.

1. The transverse, somewhat obliquely cut section of the planula of *Stylophora pistillata* (ESPER) through the slightly lower level than the stomodaeal region, 5 μ in thickness, stained with Heidenhain's iron haematoxylin and orange G. Showing the internal structure of the planula. × ca. 250. ect-ectoderm; end-endoderm; mes-mesoderm; mf-mesenterial filament; z-Zooxanthella.

2. The skeleton of polyp, 5th day of attachment, showing the septal arrangement and also an origin of calyx. × ca. 65.

3. The skeleton of polyp, 19th day of attachment, showing the columella and also the secondary modification of skeleton resulting from the development of bud. × ca. 65. c-columella; s. b-skeleton of bud.

4. The aboral surface of polyp shown in fig. 3. The septal arrangement is seen through the basal plate. × ca. 65.

5. The skeletons of two polyps of a and b, 30th day of attachment, both of them having one bud each. Showing the skeleton modified by the development of bud and also showing the peripheral upgrowth of the basal plate. In "a" only five of endosepta are developed. × ca. 32. c-columella of parent polyp, cb-columella of bud.

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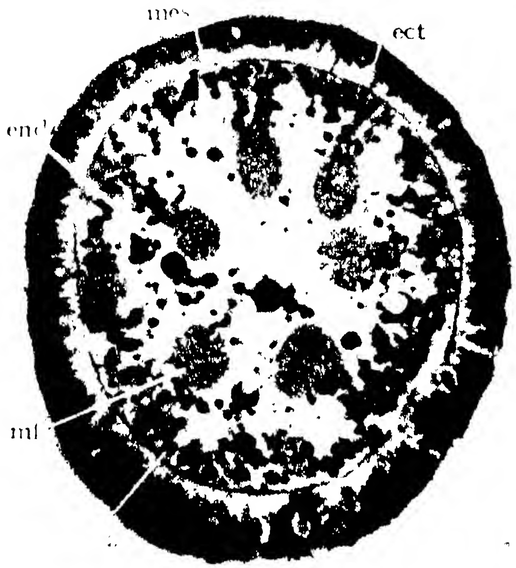

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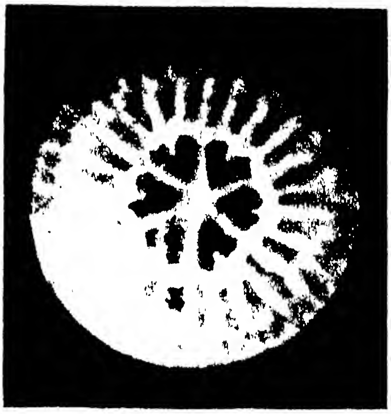

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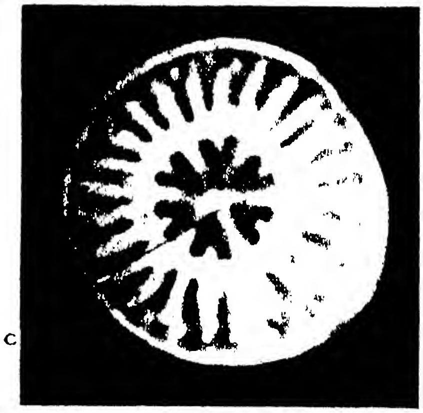

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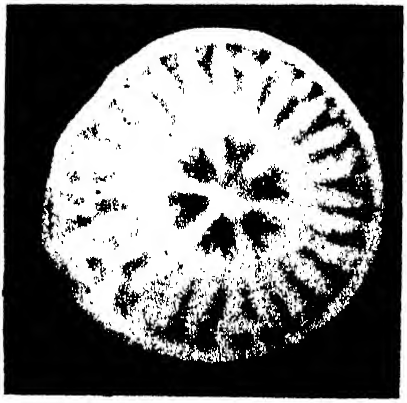

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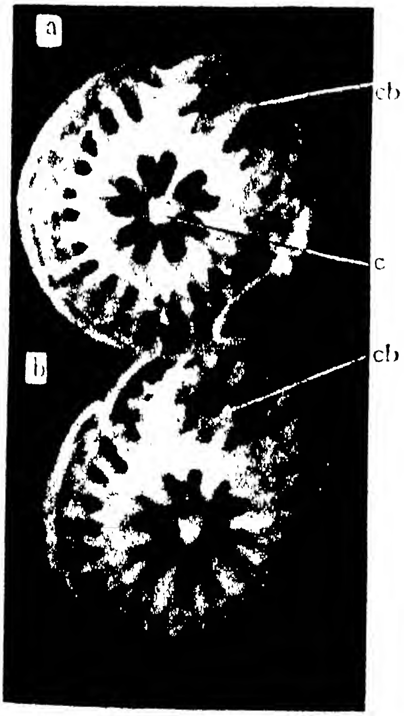

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PHYSIOLOGICAL STUDIES ON THE PIGMENTARY SYSTEM OF CRUSTACEA.

II. THE PIGMENT MIGRATION IN THE EYES OF THE SHRIMPS.*

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(With 2 figures and 1 plate)

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INTRODUCTION.

The pigment cells in the eyes of many animals exhibit movements quite like those seen in the more usual types of dermal chromatophores. These movements, however, were not discovered until about half a century ago. Although as early as 1852 H. Müller noted that the position of the retinal pigment varied more or less in different animals, it was until that Boll and Kühne (1877) independently discovered that under the influence of light and darkness, the retinal pigment of the frog moved outwards between the rods and cones and inwards toward the bodies of the pigment cells. The movement of the pigment in the eyes of invertebrates was first recorded by Exner for arthropods in 1889. Immediately following Exner's first report, there came other reports on various arthropods from Stefanowska (1890), Exner (1891), Szczawinska (1890), Herrick (1891), Kiesel (1894) and Parker (1895).

Parker published the first account of a detailed study of pigment changes in the eyes of a crustacean, *Palaemonetes vulgaris*. Owing to this and subsequent investigations, *Palaemonetes vulgaris* is the shrimp which has been most thoroughly examined in respect to the anatomy

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and physiology of its compound eyes.

Of great interest is the fact, observed rather recently, that the eye pigments in several crustaceans show a diurnal movement like that occurring in the chromatophores in some forms. (WELSH, '30, '35, '36; BENNITT, '32 b; KLEINHOLZ, '37)

Concerning the pigment cell activities in the crustacean eyes, the hormonal regulation of the pigment migration was definitely proved by KLEINHOLZ ('34, '36) in *Palaemonetes vulgaris* and by WELSH ('39) in *Cambarus*.

Recently, WELSH ('41) reported that the regularly recurring twenty four-hour cycles in pigment migration in the eyes of the crayfish under constant external conditions might be due to regular variation in the activity of inhibitory nervous centers causing a variation in the amount of hormone released from the sinus glands.

The present paper is the result of an attempt to elucidate the mechanism of pigment migration in the eyes of the shrimps, *Paratya compressa* (DE HAAN) and *Leander paucidens* (DE HAAN), with special reference to its humoral control.

The work has been carried out at the Biological Institute of the Tôhoku University, Sendai. I wish to express my cordial thanks to Prof. SHICHIROKU NOMURA for his kind directions and suggestions.

MATERIAL AND METHOD.

Two species of shrimps were used for this investigation. The first one was the common fresh water shrimp, *Paratya compressa* (DE HAAN) which had been employed for my studies on the color change. The next one was the common river shrimp, *Leander paucidens* (DE HAAN) which was about three times greater than the former in size.

In either form, the female is grater than the male, but they showed significant differences in the reactions in any of the experiments. The two sexes were used indiscriminately and with perfectly uniform results.

In order to obtain the light phase of the eyes, animals in a vessel were placed on a white background in diffuse sunlight for a period of two or three hours. At the end of this time the specimens were killed by momentary immersion in hot water (about 80° C). This served to kill the animal quickly, and at the same time to fix the eyes, so that no further change in the position of the pigment cells was possible. After immersing in CARNOY's solution overnight, their eyes were run up through the different grades of alcohol, cleared in xylol, embedded in paraffin, and cut at 10 μ. The sections, while still in the paraffin ribbon, were examined with a hand lens to find ones where the cutting had been made parallel to the axis of ommatidia.

No staining was necessary to show the position of the black pigment granules, which are insoluble in alcohol or xylol. When it was desired to bring out the details of the cells themselves, the sections were stained slightly with DELAFIELD's hematoxylin and eosin.

For the extreme dark condition, animals were placed in a light-proof box with a tight-fitting cover and left for two or twenty four hours, and then killed by pouring boiling water into the box in the absolutely dark room. The specimens were further treated in the same manner as above described.

The extracts of the eye-stalks were prepared in essentially the same manner as described in my first report on the dermal chromatophore reaction. Twenty eyes-stalks from ten animals were excised at their proximal ends and were macerated in 5 cc. of the amphibian RINGER solution, boiled, centrifuged, and the clear extract was decanted off and cooled. The extract was not filtered because of the small quantities which could be prepared at one time. As the control, RINGER's solution with no tissue constituents was used. Ten to twenty animals could be used conveniently for a single experiment. The amount of extract for one injection ranged from 0.01 to 0.05 cc.

OBSERVATIONS AND EXPERIMENTS.

1). *Structure of the Eyes of Paratya compressa* (De Haan).

For a detailed description of the finer structure of the crustacean eye and an account of the mechanics of its pigment migration, the readers are referred to the papers of Parker (1891, 1897) and of Welsh ('30, '32).

The eye of *Paratya* is a compound structure consisting of ommatidial units as in *Palaemonetes*. Composing or related with each ommatidium there are the following structures: cornea (cor.); cone (con.), rhabdome (rab.,) fenestrated basement membrane (ba. m.), and the three types of pigment cells: distal pigment cells (d. p c.=iris pigment cells), proximal pigment cells (p. p. c.=retinular pigment cells), and reflecting pigment cells (refl. p. c.=accessory pigment cells or tapetum cells). (Fig. 1.)

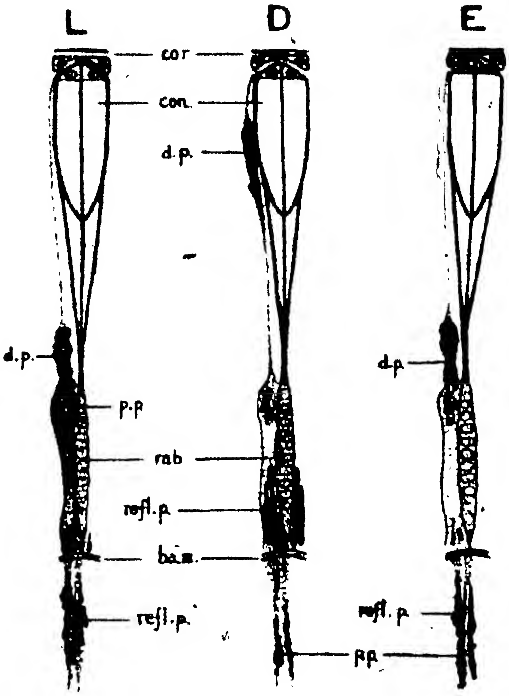

Fig. 1. Ommatidia from the eyes of *Paratya compressa*, showing the general structure and the position of the three pigments under various conditions. L, from an eye in the light condition; D, from a dark adapted eye; E, from an experimental animal which, after being adapted to darkness, was injected with stalk extract prepared from the eyes of light adapted specimens.
Cor., cornea; con., cone; d. p., distal pigment; p. p., proximal pigment; rab., rhabdome; refl. p., reflecting pigment.

In each ommatidium there are two distal pigment cells which form a collar around the cone. The crystalline cones (the dioptric system) are surrounded in sleeve-like fashion by two distal pigmen cells. These cells possess distal processes which extend to the cornea, and proximal portions seem to be continuous with the proximal pigment cells.

Proximally beyond the rhabdome the proximal cells become attenuated, pass through the fenestrated basement membrane, and, as retinal nerve fibers, connect with the first optic ganglion (lamina ganglionaris). These cells appear to be the only retinal cells to have an anatomical connection with the central nervous system.

The third kind of pigment cell is the reflecting pigment cells which are situated in the proximity of the basement membrane and are capable of moving outwards to the front face of the distal pigment cells and inwards along the retinal nerve fibers as far as lamina ganglionaris. Probably there are only one or two reflecting pigment cells in each ommatidium.

Black pigment granules are contained in both the distal and the proximal cells and the pigments must be melanin. The reflecting pigment is of a different sort and probably is guanin. When this pigment is viewed under the microscope by transmitted light, it appears yellowish or greenish brown and is barely distinguishable from the black pigment of the proximal and distal cells; examination of the section by dark field illumination, however, shows this pigment as a gleaning white granular mass.

2). *Normal Pigment Migration in the Eyes of Paratya compressa.*

The positions of the proximal pigment cells characteristic for the conditions in darkness and in light are shown in Fig. 1. The distal pigment cells which have become fully adapted to darkness have migrated distally so that they terminate approximately at the outward level of the cone. In this position, according to EXNER (1891), lateral rays of light might be admitted through one cone and then be allowed to diffuse to the receptors of adjacent ommatidia, thereby increasing the efficiency of the eye.

In the extreme proximal position, the distal pigment cells prevent overstimulation of the rhabdome by excluding lateral rays and admit only those axial light rays which enter the rhabdome by way of its cone.

The proximal pigment may be considered as moving in directions opposite to that taken by the distal pigment in response to light and to darkness. In the light, the pigment granules are found in a distal position above the basement membrane, while in a dark adapted eye the pigment has migrated proximally below the basement membrane.

The reflecting pigment apparently aids in regulating the amount of light which reaches the underlying cells. In light the pigment granules are chiefly found proximally to the basement membrane, in darkness chiefly on the distal side of it. The inward migration of the proximal pigment in the dark exposes the reflecting pigment in such a way that light which has already passed once through the rhabdome from the outside may be reflected again in order to multiply the action.

3). *Rate of Migration of the Pigment Cells in Paratya compressa.*

PARKER (1897) had determined the rate of movement of the distal and proximal pigments in the eyes of *Palaemonetes*, by making measurements on sections of eyes of animals which had been killed after varying intervals of time in the light and in the dark. By means of the same technique I found that the inward migration of the distal pigment cells in the light took place in 35~45 minutes and the outward migration in the dark in 45~55 minutes. The outward migration of the proximal pigment cells in the light took place in 20~30 minutes and the inward migration in the dark in 25~35 minutes respectively.

In respect to the exact quantitative determination of rate of the eye pigment migration, I shall study further in future.

4). *Effect of Eye-stalk Hormone on Pigment Migration in Paratya compressa.*

Two groups of tests involving the use of eye-stalk extract prepared as described above were carried out. Namely, in the first group of test, the entire procedure was reversed in order to see whether the eye-stalk hormone would induce a migration of the distal pigment similar to that produced on the pigment in the dermal chromatophore. By preliminary observation I found that the inward migration of this pigment had occurred, a maximum being reached in those animals killed after 35～45 minutes in the dark.

When sections of eyes from the dark adapted animals induced by the extract were examined histologically, the inward migration of the distal pigment was confirmed (*cf.* Plate II). The proximal pigment was unaffected by the stalk extract, since it remained in the position typical for darkness. The reflecting pigment, however, was observed to have migrated inward into the light adapted condition; that is to say, after injection of stalk extract the greater part of the reflecting pigment had moved below the basement membrane.

Two control experiments were performed for this first group of tests. The first control was uninjected and fixed at intervals during the course of 30～45 minutes in the darkness. The distal pigment in the eyes of such control showed very slight variations of its localization, but there was scarcely any significant migration of the pigment.

The second control consisted of specimens which were first adapted to darkness and then injected with amphibian RINGER solution. In none of these was there any change in the position of the pigments.

In the second group of tests, the stalk extract was prepared from *Paratya* which had been adapted to darkness. The light adapted animals injected with this extract were killed in cource of 55 minutes after that treatment and the sections of eyes tested. The pigment migration in this condition was not recognized.

5). *Structure of the Eyes of Leander paucidens* (DE HAAN) *and 'Light Effects' in the Pigment Position produced by Injection of* CO_2*-saturated Water.*

The eyes of *Leander paucidens* (DE HAAN), common river shrimp in our country, are larger than those of *Paratya* and they are good material for research of structure by reason of its transparency. The transparency of the eyes and the clear-cut boundaries of the distal pigment cells suggest the possibility of measuring the width of the transparent area between the pigment cells and the cornea, thus making it possible to obtain direct measurements of the movement of the distal pigment cells during light or dark adaptation. This will be attempted in future according to Welsh's experiment.

The structure of the eyes of this species closely resembles that of *Paratya*. From the reason the description of the structure of the eyes and behavior of the pigment will be omitted. (*Cf.* Fig. 2.)

Under the normal environmental conditions, light would cause the release of a substance activating the retinal pigment, from such an endocrine organ as sinus gland and the absence of light prevent its release, but it is far from being clear that this is the result of presence or absence of direct reflex excitation. A number factors, which are known to lower conduction

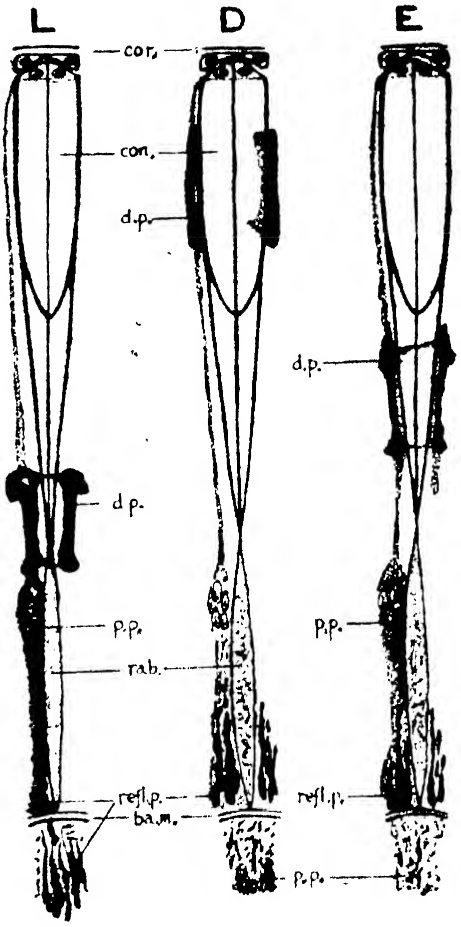

Fig. 2. Ommatidia from the eyes of *Leander paucidens*, showing the general structure and the position of the three pigments under various conditions.
L, from an eye in the light condition; D, from a dark adapted eye; E, from an experimental animal which, after being adapted to darkness, was injected with CO_2-saturated water.

rate of nerves or to decrease the activity of the nervous system, cause the release of the eye pigment hormone from the sinus gland and result in a partial or complete migration of retinal pigments to the light adapted positions, even though animals are maintained in darkness. Such factors known up to this time are low temperature, oxygen-deficiency, anaesthesis, death, and sleep or general inactivity. The effects of these on retinal pigment migration, in a large number of arthropods, have been observed by many investigators.

I have attempted to eliminate the effect of the nervous system by means of the injection of CO_2-saturated water (each dose of 0.005 cc.) into the eye-stalk directly. For this purpose, the animals were kept in dark room for 50 minutes and then the eyes were black-painted by mixture of black ink and balsam immediately before injection. The sections of the eyes treated in this way showed the outward migration of the proximal pigment, but no sign of inward migration of this pigment. There was, however, no change in the position of the accessory pigment and the distal pigment. The distal pigment was not situated in the extreme distal position by its uncomplete dark adaptation. (Cf. Fig. 2. E. and Plate I. E′ and E″) Thus the injection of CO_2-saturated water into the eye-stalk caused the every similar results as general inactivity.

COMPARISON AND DISCUSSION.

The function of distal pigment cells is the best known of the three kinds of pigment cells in the eyes of crustaceans. In bright light the distal pigment was proximal in position and border upon the pigment of the proximal pigment cells ; in darkness it was more distally located between the cones. In this manner the amount of light reaching the rhabdomes was sharply limited in bright light, whereas in dim light the pigment sheaths allow most of the light available to reach the sensitive rhabdomes. Thus the migration of the distal pigment is the adaptation of the eyes for seeing by day and night.

In general, the movements of the distal pigment cells are brought about in two different manners in different decapods. Thus in some species, like *Palaemonetes* and *Palaemon*, the cells form a sheath around the cone and rhabdome of the ommatidium and glide as a whole up and down along their axis. Both of *Paratya compressa* and *Leander paucidens* in the present investigation showed this first type of migration. The second type of migration is represented by *Astacus*, *Cambarus* and *Pagurus*. In these forms, the cell bodies remain essentially motionless at the level of the cones, and instead their pigment granules move inwards into the proximal processes of the cells and back again to the bodies of the cells.

The function of the distal pigment cells of the first type was suggested by PARKER (1897) to be the result of combined amoeboid and muscular movements, by TROJAN ('13) and MOSSLER ('15) to be akin to protoplasmic streaming. WELSH ('30) noted that each of the two distal pigment cells contains in its interior three or four fibrils which extend from the distal and of the cell body to the end of the proximal process, where they terminate at the level of the nuclei of the proximal cells. When the distal pigment cells migrate inwards under the influence of light, these fibrils shorten to one-fourth or one-fifth of their former length and thicken correspondingly. WELSH therefore supposes them to be myofibrils and responsible in the main for the inward migration of the distal pigment cell. The outward migration of the same cells in darkness can not be explained in this way, for the distal processes are not confirmed to contain any such fibrils. The mechanism of this migration is also still unknown, but according to PARKER ('32) some kind of primitive muscle-like or amoeboid action may take place within the distally elongated processes of the distal pigment cells.

The proximal cells surround the rhabdome and their pigment granules move outwards under the influence of light. Under the influence of darkness the granules migrated inwards. The consequence is that the pigments of the distal and proximal pigment cells approach one another in the light and separate in the dark. The pigment of the proximal cells behave to control the amount of light that falls upon the rhabdome; this function is carried out in correlation with the reflecting pigment cells.

In light the pigment granules of the reflecting pigment cells were chiefly found proximally to the basement membrane, in darkness chiefly on the distal side of it. The inward migration of the proximal pigment in the dark exposed the reflecting pigment in such a way that light has already passed once through the rhabdome from the outside may be reflected again in order to multiply the action. Thus it is obvious that the migration of the eye pigments are caused by light, but whether this effect is a direct action of light upon the pigment cells or not was for a long time an open question. It is equally probable that the light stimulates a receptor which in its turn induces changes which spread to the pigment. In this instance the migration of the eye pigments would represent the final stage in a reflex-circuit.

If the eye pigments are directly sensitive to light and are not stimulated simultaneously by other means or through the nervous system, the condition of the pigments in a light adapted eye of an animal should have no influence on the pigments of the other eye of the same animal, which is covered by an opaque paint. Conversely, if the nervous system is involved in the pigment migration, one eye may be iufluenced by the condition of the other. PARKER (1897) and CASTLE ('27) concluded that the illuminated eye was without effect on the position of the pigments in the covered eye, and that retinal pigment migration was independent of the central nervous system. DEMOLL ('10, '11, '17), TROJAN ('13), and BENNITT ('24) believed, however, the presence of the nervous control of pigment migration in the compound eyes, and VON FRISCH ('08) was unable to get any conclusive evidence from his experiments. This might be unable to be determined so hastily.

Of great interest in this connection is the fact, observed rather recently, that the eye pigments in several crustaceans show a diurnal movement like that occurring in the chromatophores in some species. Thus WELSH ('30) found that the distal pigment in *Macrobrachium* by day assumes the position characteristic for light adaptation and by night that characteristic for dark adaptation, independently of the light intensity. The same independent diurnal migration of the proximal pigment was found by BENNITT ('32) in *Cambarus* and by WELSH ('35) in *Peneopsis goodei*. WELSH ('35) found also a diurnal migration of the reflecting pigment cells in *Latreutes fucorum*, *Leander tenuicornis*, and *Leander affinis* and in 1936 he observed the same phenomenon in both the distal pigment cells and the reflecting pigment cells in *Anchistioides antiguensis*. Later investigation by BENNITT ('29, '32) in *Cambarus* corrected the old opinion and proved that there really is a certain effect of the illumination of one eye on the position of the pigments in the other covered eye of the same animal in *Cambarus*, *Cancer*, *Carcinides*, *Libinia*, and *Homarus*.

In consequence of the results above described, either a nervous or a humoral regulation of the migration of the eye pigments might be postulated. BENNITT ('24) suggested formerly the possibility of a humoral control of these functions, but did not regard it as important; later he ('32) and PARKER ('32) discussed the same view more seriously. On the other hand, WELSH ('30) was able to prevent the diurnal rhythm under constant illumination by ligation of the eye-stalk, the humoral hypothesis has been more and more emphasized, and finally the theory of a humoral regulation of the migration of eye pigments in crustacea was definitely proved by KLEINHOLZ ('34, '36) in *Palaemonetes vulgaris*.

The action of the pigment-activating substance is not limited within the species; extracts of the eye-stalk of *Cancer irroratus*, *Libinia dubia*, *Uca pugilator*, and *Carcinides maenas*, when injected into dark adapted

Palaemonetes, also affected the migrations of distal and proximal pigments. It was seen also in my experiment with *Paratya compressa*. With higher or lower degree of probability, the eye pigment migration is supposed to be connected with the eye-stalks in higher crustaceans. The only humoral function which can unquestionably be referred to a definite incretory organ in the eye-stalk is the pigment activating hormone which would have its source in the sinus gland discovered by HANSTRÖM.

Recently WELSH ('41) reported on the innervation of the sinus gland in the eyes in *Cambarus bartoni*. According to his work, the motor fibers from one of the oculomotor nerves go to the region of the sinus gland, and fibers from the medulla terminalis and from the supra-oesophageal ganglion join to form the sinus gland nerve which can be traced into the tissue of the gland. It is suggested that tonic inhibitory centers in the medulla terminalis or supra-oesophageal ganglion normally prevent the release of the retinal pigment hormone. Stimulation of the eye by light reduces or abolishes the activity of these inhibitory centers and allows the release of hormone. The results of the eye pigment migration with CO_2-saturated water in *Leander pauciden*s may be offered for this evidence as well as the observed effects of chloretone anaesthesia, O_2-lack, low temperature and general inactivity by some authors, because these factors, which would tend to lower the activity of nervous system, cause a migration of retinal pigment to or toward the positions characteristic of the light adaptation, even though animals are kept in the dark.

Considering from my own result and from those facts above mentioned, it may be probable that the light adapted positions of eye pigments would represent the active state of humoral control or inactive state of the inhibitory nervous centers; the dark adapted positions of eye pigments would correspond to resting state of humoral control.

SUMMARY AND CONCLUSION.

1. The common fresh water shrimps, *Paratya compressa* (DE HAAN) and *Leander paucidens* (DE HAAN) were used in the study of the pigment migration in the eyes.

2. An ommatidium is supplied with two pigment cells, each having a distal and a proximal processes. These cells form a collar around the cone and move inwards and outwards under the influence of light and darkness, thereby aiding in regulating the amount of light reaching the rhabdomes.

3. The movement of the reflecting pigment was clearly recognized. In light the pigment granules were chiefly found proximally to the basement membrane, in darkness chiefly on the distal side of it.

4. The inward migration of distal pigment cells in the light took place in 35~45 minutes and outward migration of these pigment cells in the dark in 45~55 minutes.

5. The outward migration of proximal pigment cells took place in 20~30 minutes and inward migration of this pigment cells in the dark in 25~35 minutes.

6. The injection of eye-stalk extracts of light adapted *Paratya compressa* to the dark adapted individuals induced the inward migration of the distal pigment and reflecting pigment just as in light adaptation.

7. The stalk extract prepared from dark adapted *Paratya* had no influence on the pigment migration of eyes of light adapted individuals.

8. The injection of CO_2-saturated water to the eye-stalks of dark adapted *Leander paucidens* showed the outward migration of the proximal pigment.

9. It may be probable that the light adapted position of eye pigments represents the active state caused by humor or inactive state of inhibitory nervous system and the dark adapted position of eye pigments would be inactive state of pigment cells in the absence of humoral agent.

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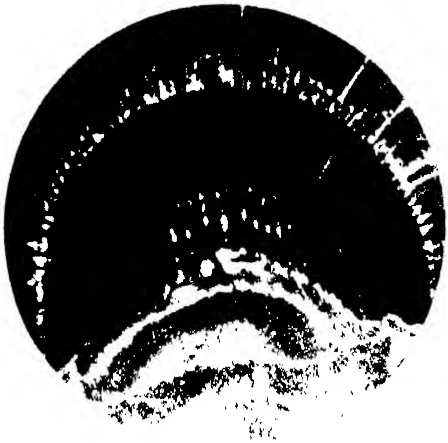
EXPLANATION OF PLATE II.

L, D and E—Longitudinal sections of the eye of *Paratya compressa*, showing the position of the three pigments under various conditions: L, from an eye in the light condition; D, from a dark adapted eye; E, from an experimental animal which, after being adapted to darkness, was injected with stalk extract prepared from the eyes of light adapted specimens.

E′ and E″—sections of the eye of *Leander paucidens*.



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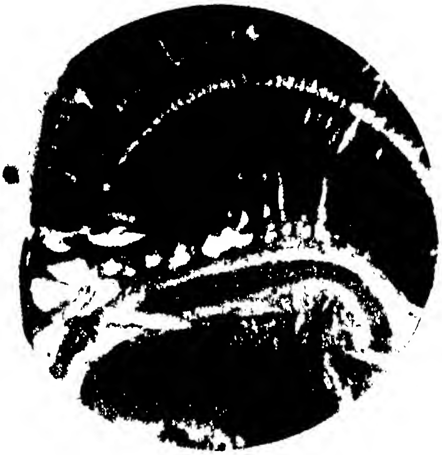

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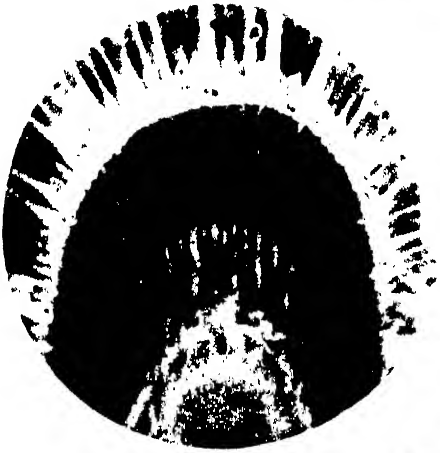

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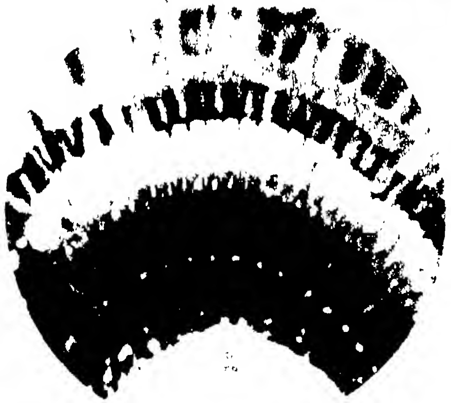

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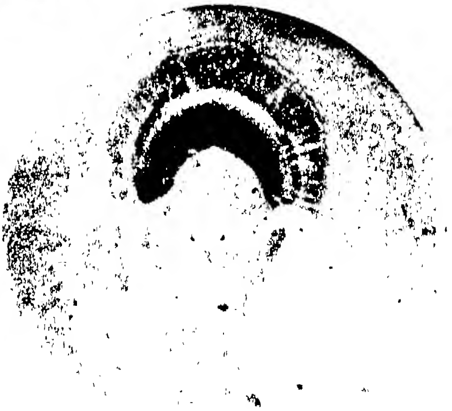

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CORRECTIVE EFFECT OF α-DINITROPHENOL ON THE LITHIUM LARVA OF THE SEA URCHIN.

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The effect of lithium salts on the sea urchin's embryo was observed for the first time by HERBST (1892). The distortion of the gastrulation is caused by decreasing the prospective amount of the ectoderm of the larva cultured in sea water containing a small amount of LiCl. Recently the mechanism of the exogastrulation in the sea urchin has been studied by the Scandinavian embryologists, RUNNSTRÖM (1935), HÖRSTADIUS (1928) and LINDAHL (1936). Their opinion may be classed as one of the gradient theories, and according to them the exogastrulation is the result of the decrease of the animal gradient in comparison with the vegetative gradient. The biochemical basis of this hypothesis is in the facts that the effective concentration of LiCl inhibits the oxygen consumption of the larva, and that there are some reagents exhibiting an antagonistic, animalizing effect upon LiCl. And, moreover, the animalization can be caused in two directions, the one is to decrease the vegetative gradient, as well as the vegetative metabolism, and the other is to recover from the inhibition of the animal gradient, as well as the animal metabolism, of the lithium larva. Accordingly, the metabolism of the animal pole of the egg is qualitatively different from that of the vegetal pole, and the former is the glycolysis according to LINDAHL (1936).

Recently, NEEDHAM and NEEDHAM (1940) have tested the exogastrulation-effect of the reagents, which are known as the inhibitor of the glycolysis of the tissues of vertebrates, with the purpose of ascertaining

LINDAHL's hypothesis, that the animal gradient is the concentration gradient of the glycolysis. The results were negative in all three reagents, iodacetate, glyceraldehyde and phloridin. Up to the present time, unfortunately, the effect of LiCl on the metabolism of the cell is not thoroughly clear, as far as the writer is aware, and consequently it will be necessary to accumulate many unknown fundamental facts concerning the effect of lithium on the embryogenesis of the sea urchin.

The experiment was carried out in the summer of 1941 at Misaki Marine Biological station. The material used was a sea urchin, *Anthocidaris crassispina* (A. AGASSIZ). This species is very useful for the study of the exogastrulation, because the development is very rapid; that is, 50 minutes after fertilization the first cleavage begins, the cleavage interval being 25 minutes, and after 6 hours the swimming blastula hatches out, after 12 hours the gastrulation begins, and after 24 hours the larvae become plutei.

TABLE 1. The effect of LiCl on the fertilized egg of *Anthocidaris crassispina.*

Amount of M/2 LiCl in sea water	6 hours	10 hours	12 hours
13 %	blastula	bottom swimming blastula	disintegrated
9 %	"	"	inactive blastula
6.5 %	"	top swimming blastula	sluggish blastula
4.8 %	"	"	"
3.4 %	"	"	gastrula

The effect of LiCl on this species is shown in table 1. In 13 per cent the egg could develop normally for 6 hours, but after 10 hours the larva lost its normal activity, and 12 hours after all larvae disintergrated. In the concentrations higher than 4.8 per cent the activity of the larvae was abnormally low after 12 hours. But in 3.4 per cent the normal gastrula developed. Thus in this species, the specific effect of lithium ions is indistinct in the prolonged action of a dilute solution of LiCl. On the other hand, when the fertilized eggs were first immersed for six hours in a relatively concentrated solution of LiCl in sea water and then cultured farther in ordinary sea water, the typical effect of lithium could be odserved without losing the activity of the larva. For instance, the eggs thus treated with 13 per cent of M/2 LiCl in sea water became holoentoblastiae, and with 9 per cent and 6.6 per cent they became exogastrulae and exoentogastrulae respectively. In 4.8 per cent the normal plutei were formed. Accordingly, in the following experiments the lithium sea water as well as a mixture of lithium sea water and α-dinitrophenol was used for the first six hours after fertilization, and after this the eggs were cultured in ordinary sea water.

Five parts of saturated aqueous solution of α-dinitrophenol were mixed with 95 parts of 10 per cent lithium sea water. Ten minutes after fertilization the eggs were put into this mixture and after 6 hours they were transfered into ordinary sea water After 24 hours plutei with short arms were formed. The control, 10 per cent lithium sea water, showed the typical exogastrulation. That is, the effect of 10 per cent lithium was completely cancelled. But, on the other hand, α-dinitrophenol did not show the animalizing effect, when it was singly added to ordinary sea water. These facts show that the effect of α-dinitrophenol is not comparable with that of NaSCN and of SO_4-free sea water. According to LINDAHL (1936) and HERBST (1897) the last two reagents show the animalizing effect even when they are singly used, and the development of the ectoderm is abnormally accelerated. But, in α-dinitrophenol this was not the case. And here, the mode of effect of α-dinitrophenol will be tentatively designated the corrective effecct.

In the concentration of α-dinitrophenol mentioned above the cleavage of the egg was completely inhibited as long as the eggs were in this medium, and soon after return to sea water it recovers the normal process. This shows that the eggs became insensitive to lithium by being inhibited their cleavage. The inhibiting effect of α-dinitrophenol on the cleavage is comparable neither with colchicin nor with other narcotica, because colchicin inhibits the development of the cytaster and spindle, whereas in *α*-dinitrophenol the well developed cytaster and the spindle were observed but the division of the chromosomes were inhibited.

SUMMARY

The vegetalizing effect of lithium ions on the development of the sea urchin's eggs is cancelled by *α*-dinitrophenol, when *α*-dinitrophenol is added to the lithium sea water. The mode of action of *α*-dinitrophenol is distinguished from the animalizing effect of NaSCN or that of SO_4-free sea water in the points that *α*-dinitrophenol did not show the animalization of the eggs when used alone, and that it insensitizes the eggs to lithium by inhibiting the cleavage.

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CONTRIBUTION TO THE BIOCHEMISTRY OF THE CORAL

IX. INORGANIC COMPOSITION OF THE SKELETON OF THE CORAL, *FUNGIA ACTINIFORMIS* VAR. *PALAWENSIS* DÖDERLEIN

BY

KEIZO HOSOI

(Received August 23, 1946)

While some chemical data are recorded of the skeleton of corals in literature, we are almost ignorant of the chemistry of the polyp. A good knowledge of biochemical organization of the polyp is so much important as that of the former, particularly in understanding the mechanism of skeleton formation. Therefore, the study on the chemical composition of the coral was firstly undertaken to fill the gap.

The eight papers of biochemical study on the coral, *Fungia actiniformis* var. *palawensis* DÖDERLEIN have been so far published in the Palao Tropical Biological Station Studies. The 1st paper reported on the occurrence of glycogen and its content in the polyp (1), the 2nd one on the occurrence of sugar and its content in the polyp (2), the 3rd one on the copper content of the polyp and the skeleton (3), the 4th one on the iodine in the polyp (4), the 5th one on the distribution of nitrogen in the protein of the polyp (5), the 6th one on the calcium and carbonate in the skeleton (6), the 7th one on the fungiasterol, a new sterol, of the polyp (7), and the 8th one on the contents of cystine and amino acids in the polyp (8). As described above, biochemical studies on the coral, which were carried out by the present writer, dealt mainly with the chemical composition of the coral, *Fungia actiniformis* var. *palawensis* DÖDERLEIN.

The skeleton of the coral studied in the present work is remarkably rich in inorganic constituents, but poor in organic ones. A great portion of inorganic constituents in the skeleton consists of Ca and CO_3. The former amounts to 38.39 % and the latter 52.62 % (6). The skeleton was found to be remarkably poor in copper and its content was 0.074-0.090 mg %. The present paper deals with the other inorganic composition of the skeleton. That is, potassium, sodium, magnesium, chlorine, phosphate, sulphate and silica were determined.

METHOD AND RESULTS

For the analysis the skeleton was washed with redistilled water and powdered. After it had been dried to constant weight at 110°, an aliquot portion was weighed out and dissolved by the addition of dilute nitric acid. The insoluble matters which consisted probably of organic matters, remained little in quantity in the solution and were destructed completely by heating with nitric acid. The nitric acid solution of inorganic and organic matters thus obtained was analysed as follows. Potassium and sodium were determined gravimetrically as chloride and the potassium as chloroplatinate. Magnesium was estimated by the gravimetric method as magnesium pyrophosphate. The molybdic method was used for the determination of phosphate. Chlorine was estimated by the Volhard method. Sulphate and silica were

TABLE 1. Inorganic composition of the skeleton (%)

Material	Na	K	Ca	Mg	Fe	Cu	
Fungia actiniformis var. *Palauensis* DODERLEIN	0.124	0	38.39	0.091	—	0.00082	HOSOI
Gorgonia sp.	Trace	—	38.51	1.92	0.74	—	VOGEL (9)
	Cl	CO_3	PO_4	SO_4	SiO_2	H_2O	
Fungia	0.038	52.62	0.247	0.464	0.362	0.44	HOSOI
Gorgonia sp.	Trace	39.85	—	0.37	—	5.31	VOGEL

determined by the gravimetric method in the usual way. The results so obtained are shown in the following Table.

Potassium was not found to be present in the skeleton by the gravimetric method used. The other inorganic substances determined in the present work were very little in quantity in comparison with Ca and CO_3. The skeleton of *Gorgonia* as well as that of *Fungia* consists mainly of Ca and CO_3. In the calcic skeleton of stone corals, mineral constituents and organic substances have been reported to be 90.6–97.9 % and 2.11–9.43 % respectively. More than 95 % of the mineral constituents consists of calcium carbonate, aragonite. Among fossil stone corals some skeletons consist of calcite. In the skeleton of stone corals, phosphate and fluoride are found to be 0.3-2.6 % and magnesiun carbonate is present in a very amall amount. Mineral constituents in the skeleton of *Fungia* amount to more than 92.77 %.

SUMMARY

Potassium, sodium, magnesium, chlorine, phosphate, sulphate and silica in the skeleton of *Fungia actiniformis* var. *palauensis* DÖDERLEIN were determined.

At this place I wish to express my hearty thanks to Dr. S. Nomura, Professor of Animal Physiology, Biological Institute of the Tohoku Imperial University, for his kind revision of the present paper.

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CONTRIBUTION TO THE BIOCHEMISTRY OF THE CORAL

X. A NOTE ON THE COMPARATIVE PHYSIOLOGY OF THE CORAL, *FUNGIA ACTINIFORMIS* VAR. *PALAWENSIS* DÖDERLEIN.

BY

KEIZO HOSOI

(Received August 23, 1946)

INTRODUCTION

As described in the first paper, a good knowledge of biochemical organization of the polyp is no less important than that of the skeleton, particularly in understanding the mechanism of skeleton formation. Therefore, a series of study was firstly undertaken to fill the gap for which it had been so far not reported. As regards the chemical constitution of the body of Octocorallia, studies on the constituents of living material are within the limited range. The elements of the skeleton of Octocorallia are well-known for they might be the most favourable material for analytical research. As some chemical data (1-9) have been obtained in the series of biochemical studies on the coral, *Fungia actiniformis* var. *palawensis* DÖDERLEIN, the present writer wishes to describe briefly some correlation on the comparative physiology of the coral.

I *Comparative Physiology of The Polyp of The Coral.*

THE CHEMICAL COMPOSITIONS

The chemical composition of the polyp of the coral, ***Fungia actiniformis*** var. *palawensis* DÖDERLEIN was determined as follows: water 83.66 %, ash 1.72 %, carbohydrates 0.14 %, fats 3.78 % and proteins 10.70 %. The chemical compositions of the bodies of various Coelenterata are compared in Table 1.

TABLE 1.

species	organic substance %	ash %	water %	observers
Actinia equina (L.)	15.55	1.76	83.19	KRUKENBERG (10
Anemonia sulcata (PENN.)	10.68	1.60	87.56	"
" "	9.53	3.27	87.2	PÜTTER (11
Sagartia troglodytes JOHNST.	20.88	2.28	76.84	KRUKENBERG (10)
Cerianthus membranaceus (SPALL.)	11.58	1.71	87.71	"
mean value	13.64	2.12	84.5	
Fungia actiniformis var. *palawensis* DÖDERLEIN	14.62	1.72	83.66	HOSOI 7)
Aequorea coerulescens	0.65	2.94	96.43	KOIZUMI and HOSOI (12, 13)
Cyanea capillata	0.70	2.85	96.47	"
Dactylometra pacifica	0.75	2.85	96.41	"
Cribrina artemisia	22–25	1.76	73–76	KOIZUMI and HOSOI (13)
Metridium dianthus	17–21	1.8	77–81	"
Anthopleura sp.	20–24	1.7	74–78	KOIZUMI and HOSOI (13)
Diadumene luciae	—	—	73–75	"
sea water	0.58	2.86	96.57	KOIZUMI and HOSOI (12, 13)

As shown in Table 1, the percentages of the chemical composition of the actinozoa is different from that of the medusae. According to KOIZUMI and HOSOI (12) the percentages of water, of ash and of organic substances of the central part of the exumbrella of the medusae are remarkably alike to those of sea water in which they live. They stated also that as regards the amount of each of the constituents, taking the sodium as the standard (the sodium content of the annual mean sea water is practically the same) the calcium and the magnesium are practically equal to or slightly less, chloride ion slightly greater, potassium considerably greater and sulphate considerably less than in sea water. The sum of cations (or anions) of the medusae is practically equal to that of the sea water (annual mean), and the sum of cations of the medusae is, within the experimental error, equal to that of anions, and the total sum of ions of the medusae expressed as gram-ions is practically equal to that of the sea water. On the other hand the percentages of water, of ash and of organic substances of the body of the actinozoa are considerably different from those of sea water. From the standpoint of active absorption, the function of regulating the permeability in the actinozoa is considered to be much more developed than in the medusae. According to HOSOI (14) the sea-anemone kept in the sea water, in which the concentration of calcium ion was increased by the addition of isotonic calcium chloride solution, the diffusion of water was not very evident, but the calcium content of the animal tissue definitely was increased. If the animal was returned into the normal sea water, the calcium content returned to the normal level. The sea-anemone, including both animal surface and coelenteron, is permeable to calcium ion. The fact that the calcium and the magnesium of the sea-anemone are practically equal to or slightly less than in sea water, is of great interest and importance in considering the experiment on the permeability of calcium in the sea-anemone and the skeleton formation of the coral.

1. *Glycogen*

Glycogen occurs very generally in vertebrates and is also widely distributed in invertebrates. The occurrence of glycogen has been reported in various kinds of invertebrates by many authors, although in most cases the statements seem to be based on insufficient data. Glycogen is found in all cells that have the possibility of growth. Glycogen, or some substance similar to it, occurs in many of the lower animals. Paramylum occurs in the flagellate Euglena. Glycogen, which was identified with the glycogen of liver, was found in protoplasm of the Myxomycetes Aethalium. A substance very similar to glycogen has been found in *Paramoecium*, *Opalina*, *Bursaria* and *Vorticella*. Paraglycogen occurs abundantly in the gregarines. None of these substances has been found among the porifera. How these animals and echinoderms store up carbohydrates is uncertain. Among the worms, *Lumbricus*, *Taenia*, and *Ascaris*, glycogen appears. It is well-known that among the mollusca glycogen reaches its highest concentration. It is found in *Cardium* to the extent of 14 % ; in oyster, 9.5 % ; in *Pecten* muscle, 2 to 2.5 % ; in foot of *Helix*, 3 3.5 % of fresh weight. Among arthropods, large masses of glycogen occur in fly larvae. Among coelenterata, many species of the hydrozoa and *Actinia equina* are said to contain glycogen. But there is no report about the glycogen content of true corals, except that some authors stated colour reactions and suggested the presence of carbohydrates or its derivatives. Glycogen has been isolated for the first time by the present writer from the polyp of the coral, *Fungia actiniformis* var. *palauensis* DÖDERLEIN. It was identified as glycogen of higher animals by the examination of physical and chemical properties and decomposition products. The glycogen content of the polyp amounts to about 0.05 %. The fact that carbohydrates reserved are found in certain organs of the polyp partly in the form of glycogen in order to serve as energy sources in the case of necessity, seems to indicate that glycogen has the same physiological functions in corals as in higher animals.

2. *Reducing Sugars*

Concerning reducing sugar in corals we are informed no better than about glycogen. There is a little evidence indicating the use of sugars by the protozoa. Apparently no one has found, among the protozoa, enzymes which are capable of digesting sugars. Among the porifera, coelenterata, and in all higher groups, however, such enzymes are found. Sugar supplies the energy needed for muscular and other work. If there be an excess of sugar over the amount used by the body as a supply of energy, it may be stored up as glycogen. As has been sated above, sugar plays an important part in the metabolism. Therefore, the present author studied the occurrence of reducing sugar in the polyp of the coral, and it was found to be present in *Fungia actiniformis* var. *palawensis* DÖDERLEIN. As to the nature of reducing sugar no definite statement can be made now. The amount of carbohydrates was about 0.14 % of the polyp.

3. *Copper Content*

It is well-known that marine animals often contain considerable quantities of copper (15). Among marine invertebrates the bloods of some of the molluscs and crustacea contain haemocyanin, a respiratory pigment, which functions as an oxygen carrier. The copper contents of haemocyanins of *Sepia* and Crustacea, *Octopus* and *Limulus* are 0.328 %, 0.38 % and 0.28 % respectively. Among coelenterata the copper content of *Actinozoa metridium* and sea-anemone is reported to be 0.1 mg. per 100 gr. of fresh weight (16). The copper content of the body of *Anemonia sulcata* (PEEN.) is 0.02 % of the dry substance (17). The copper content of the polyp of *Fungia actiniformis* var. *palauensis* DÖDERLEIN was determined by the cryogenine procedure described by SARATA (18). Its content amounts to 0.16 mg. per 100 gr. of the fresh material and about 0.01 % of the dry basis. Its content is lower than that of the oyster, but is of almost the same order as those reported by recent workers for marine invertebrates. Copper is now generally considered to be an important and essential constituent of the living organism and also essential for the growth and metabolic processes. The fact that the coral contains almost the same amount of copper as that of many other marine invertebrates indicates that the copper of the coral may possibly have the same physiological functions of copper as above stated.

4. *Iodine content*

It is reported that a relatively large amount of iodine is found to be present in the organic skeleton-substance of some species of the coral. Iodine is an essential constituent of living organisms. Of coelenterata, *Obelia longissima* (19) and *Aurelia flavidula* (20) are reported to contain iodine. KRUKENBERG (21) stated that the polyp of Octocorallia contains a trace of iodine. According to C. T. MORNER (22) the polyp of Octocorallia is free from iodine. The present writer determined firstly the iodine content in the protein of the polyp with the method of TREVORROW and FASHENA (23, 24). Its content is about 0.46 mg. per 100 gr. of the polyp. The material subjected to the determination of iodine may possibly not contain inorganic iodine compounds. Therefore, the iodine estimated may possibly be present in organic combination. The total content of iodine in the polyp was not determined.

5. *Nitrogen Content*

While the distribution of nitrogen in the organic substance of the skeleton of the coral, gorgonin, has been reported by STARY and ANDRATSCHKE (25), who determined nitrogen by the method of van SLYKE, there is no report about the distribution of nitrogen in the protein of the polyp of the coral. Therefore, the author determined the distribution of nitrogen in the protein of the polyp of *Fungia actiniformis* var. *palauensis* DÖDERLEIN with the method described by UTINO (26).

The content of proteins including amino acids is 10.70 % of the polyp. The free amino acids content of the polyp was determined by SÖRENSEN's formal titration method. Its content as glycocoll was about 0.12 % of the fresh material. The cystine content of the acid hydrolysate of the polyp was determined by the modification of the OKUDA's iodine method (27, 28, 29). Its content amounts to 0.189 %

of the fresh material. The nitrogen content of the polyp amounts to about 15.3 %.

6. *Sterols and Fungiasterol*

Sterols are important and essential substances for the living organism and their functions have an intimate relation with the activities of the cells. Cholesterol is universally present in all the vertebrates and also is widely distributed in invertebrates. Some kinds of sterols closely related to cholesterol are present in some kinds of invertebrates. Cholesterol is present in sea-anemones, but there is no report about the presence and chemical properties of sterols in the polyp of the coral. The present writer have studied the sterol of the polyp of *Fungia actiniformis* var. *palauensis* DÖDERLEIN. The sterol which was not identified with the known sterols, has been isolated for the first time from the polyp. It is considered as a new zoosterol by the examination of its properties. The author has suggested the name "*Fungiasterol*." Its contents in free and ester form were 0.061 % and 0.086 % of the polyp respectively. The physical and chemical properties of the sterol isolated indicate that it may possibly has the same physiological function as cholesterol.

7. *The Other Constituents*

The following substances are proved to be present in *Actinia equina* (L.). That is, tetramine, adenine, methylpyridilammonium hydroxide and aktinin. The latter is the base which has been so far found only in *Actinia* and is probably identical with well-known stachydrin obtained from the plant kingdom (30, 31, 32) Lipoids are proved to be present in the follwing species.

Actinia equina (L.) (10), *Anemonia sulcata* (PENN.) (10, 11), *Cereus dunculatus* PENN. (33), *Celliactis parasitica* GOSSE (10), *Sagartia troglodytes* JOHNST (10), *Diadumene luciae* (VERR.) (34), *Cerianthus* spec. (10).

As described above the chemical data of the polyp of the coral are very meager. The present writer contributed to some extent to the biochemistry of the polyp of the coral by carrying out a series of study on the coral.

II Comparative Physiology of The Skeleton of The Coral

THE CHEMICAL COMPOSITIONS OF THE SKELETON OF THE CORAL

There are some chemical data on the skeleton of the coral. VOGEL (38) gave the following analysis for the skeleton of Gorgonaria: CO_2 27.5, lime 50.5, magnesia 3, red iron oxide 1, H_2O 5, organic substance 0.5, calcium sulphate 0.5, sodium chloride a trace. VALENCIENNES (35) named the horn-mass of the axis of Gorgonaria cornein. KRUKENBERG (21) prepared cornein from *Rhipidogorgia flabellum* and found the occurrence of leucine and glycocoll in the hydrolysate of cornein which was obtained by heating with dilute sulphuric acid, but he did not find the presence of tyrosine and reducing sugars in the hydrolysate. Indol was isolated from cornein in the fusion with potash. KRUKENBERG studied the red pigment of the axis of *Corallium rubrum*. According to DRECHSEL (36) the axis of *Gorgonia cavolini* showed the following results: C, 49.4, H, 6.8, N, 17.2, I, 7.8, Cl, 2.2. Also he studied the iodine compound in the skeleton of *Gorgonaria cavolini* and reported that the organic substance of the skeleton, gorgonin, contained 9.01 % of iodine and 4.54 % of sulphur. Iodine occurs particularly in di-iodotyrosine of iodogorgonic acid which

TABLE II

Matetial	Iron oxide	Magnesium carbonate	Calcium carbonate	Calcium phosphate	Calcium sulphate	organic substance H O
Iris Hippuris		6,36 6.36				32.04
Pennatula spec.			44.25 53.57	23.7 16.3	1.38	30.43 9.83
		$\underbrace{\qquad\qquad}_{81}$				
Corallium rubrum	1 4 25	3.5 2.13	83.25			7.75

amounts to 0.68 % of the axis of Gorgonaria. In the basic hydrolysate of cornein, arginine and lysine were obtained. MENDEL (37) found only 1.7-0.28 % of iodine in *Gorgonia flabellum*, *Gorgonia acerosa* and *Plexaura flexuosa*. He gave the further analysis as shown in Table 11.

MORNER (22) regarded gorgonin as an albumoid-like substance which was incrusted with calcium carbonate, calcium phosphate etc. The sea water contained: 0.0002 % I, 0.008 % Br, 2.07 % Cl. According to MORNER (22) gorgonin contained in maximum: 6.9 % I, 4.2 % Br, 0.3 % Cl. MORNER reported the content of iodine, bromine and chlorine in the axis building Octocorallia to which Gorgonaria and Pennatularia belong. According to MORNER, DRECHSEL's iodogorgonic acid is 3.5 di-iodotyrosine containing the iodine content of 38.7 %. MORNER found the bromogorgonic acid, namely 3.5 dibromotyrosine, in his study on *Primnoa lepadifera* L. (= *P. resedaeformis* GUNN.). Furthermore it was found to contain: tyrosine, glycocoll, alanine, leucine, asparagic acid, glutamic acid, oxalic acid. From the various fractions of gorgonin the following substances have been so far obtained in pure form: lysatin, lysine, tyrosine, leucine, glycocoll, alanine, asparagic acid, glutamic acid and oxalic acid.

1. *The Inorganic Compositions*

The skeleton of *Fungia actiniformis* var. *palauensis* DÖDERLEIN is remarkably rich in inorganic constituents, but scarce in organic substances. The inorganic composition of the skeleton analysed by

TABLE III

Inorganic composition of the skeleton of *Fungia actiniformis* var. *palauensis* DODERLEIN, (%).

Na	0.124	PO_4	0.247	H_2	0.44
Mg	0.091	Cl	0.038		
Ca	38.39	SO_4	0.464		
Cu	0.00082	SiO_2	0.362		
		CO_3	52.62		

the present writer is shown in the Table III.

A large portion of the skeleton of *Gorgonia* is CO_2 and lime as well as *Fungia*. In the calcic skeleton of stone corals, mineral constituents and organic substances have been reported to be 90.6–97.9 % and 2.11–9.43 % respectively. More than 95 % of the mineral constituents consists of calcium carbonate. In the skeleton of stone corals, phosphate and fluoride are found to be 0.3–2.6 % and magnesium carbonate is present in a very small amount. Mineral constituents in the skeleton of *Fungia actiniformis* var. *palawensis* DÖDERLEIN amount to more than 92.77 %.

2. *The Organic Compositions*

As described above the chemical data of the skeleton of the coral, particularly of the organic substances of the skeleton are to some extent recorded in literature. But there is no report about the inorganic composition of the skeleton of *Fungia actiniformis* var. *palawensis* DÖDERLEIN. The organic composition of the skeleton is not yet studied in this series of investigation. The chemical analysis of the inorganic composition of the skeleton was firstly undertaken in clearing the mechanism of skeleton formation.

SUMMARY

The comparative physiology of the coral, *Fungia actiniformis* var. *palawensis* DÖDERLEIN, was described to some extent with some chemical data obtained in the series of biochemical study on the coral (1–9).

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At this place I wish to express my sincere thanks to Dr. S. NOMURA, Professor of Animal Physiology and Dr. T. KOIZUMI Assistant Professor of Biochemistry, Biological Institute, the Tohoku University, for their kind direction and advices.

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BOTANICAL STUDIES OF BOG LAKES IN A VOLCANIC REGION WITH SPECIAL REFERENCE TO LACUSTRINE BACTERIA.[1]

PART I. BASINS, WATER AND VEGETATION.

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INTRODUCTION

During the past several decades Japanese scientists have made a great deal of contribution to the developments in knowledge of the lakes in the country. Nevertheless we have still lots of important problems to pursue. Among them are two themes dealt with in this paper, namely; first, the relationships of the dystrophy to the volcanic action, and secondly, the lake bacteria.

Amongst a variety of lakes in Japan acid waters are of intense interest because they are closely linked with the volcanic action particularly characteristic of her. Not outstanding acidotrophic lakes alone, but also dystrophic ones found in volcanic regions may be developed under the influence of the volcanic activity. In reality it is found to be the case in bog lakes on Mount Hakkôda to be mentioned below.

On seeing the limnological literature of this country, one would notice that, whilst the general animal and vegetable world in Japanese lakes has received a good deal of attention up to the present, the bacterial populations have so far been ignored, little being known of their nature. It is deemed desirable to fill this gap left in our limnology. We cannot discuss the true economy of lakes without microbial activities being reckoned with.

The aim of this work has been to disclose, in the first place, the genesis and features of bog lakes in the volcanic region of Mount Hakkôda; secondly, deal with their aquatic vegetation from an ecological viewpoint; and lastly, work especially upon myriad bacteria leading a masked existence therein. I have been working since the year 1939, availing myself of the opportunities of staying almost every summer in the Mt. Hakkôda Botanical Laboratory of Tôhoku University.

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[1] Contributions from the Mt. Hakkôda Botanical Laboratory. No. 31.

This article has partly been and will partly be published in the Japanese language in "Seitaigaku-Kenkyû".

I wish to record my indebtedness to the Department of Education for awarding me grants from the Scientific Research Expenditure.

I should like to tender my very grateful thanks to Prof. Dr. Y. Yoshii, by whose kind help I was enabled to do this work.

LAKE BASINS AND LAKE WATER

Situation. At the very northern end of the mountain range running lengthwise in the midst of the Main Island of Japan, Mount Hakkôda, an extinct volcano, rises to the height of 1585 m. It is characterized by a number of bogs found here and there, and the bogs are interspersed with a vast number of pools of a dystrophic type. All these pools are alike in the way in which they have been developed, filled equally with brown water and inhabited by the same kinds of organisms.

In one of the bogs at an altitude of 980 m. lie four dystrophic waters with copious growths of a water lily, bearing graceful flowers in summer-time. It is because of this that they are collectively named Suiren-Numa—literally "water-lily ponds". Among those by far the largest (20 a.) and deepest (2.5 m.) is Naga-Numa, with which I have worked principally.

Our knowledge of the salient features of the bogs in this mountain is due to Professor Yoshii[1], who, working particularly with a bog called Kenashitai, has brought out that these level bogs developed directly upon volcanic ash are dominated by Moliniopsis japonica and Eriophorum vaginatum among a number of olygotrophic plants thriving there, Sphagnum being relatively few, and that they fall into the category of primary intermediate moor in Koppe's sense of the word. Of the very bog immediately round Suiren-Numa in particular a detailed account of the vegetation has been published by Iwata[2]. As to the pools in question themselves, no investigations in great detail have ever been made before, though brief observations limited to a single visit are not lacking.

Morphology and Hydrology of Basins. The morphometric data of the four basins are set out in Table I. They are devoid of inlets, while Naga-Numa and Suiren-Numa, both lying on the lowest level, have each an outlet. These pools are confined within a boggy area (nearly 1 ha.) surrounded by bushes

Table I. Morphological data of basins and features of the surface water.

	Maximum depth m.	Area sq. m.	Water colour on Pt-Co standard	pH[1]	Dissolved[2] oxygen %	Permanganate consumption mg. per l.
Naga-Numa	2.5	1970	100	4.9	90	40.1
Suiren-Numa[3]	1.5	730	80	5.2	88	40.4
Hyôtan-Numa	1.4	290	50	4.9	99	17.2
Tsuki-Numa	1.0	200	200	4.9	81	51.8

1) At sunny midday. 2) In a clear afternoon. 3) In the narrower sense.

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[1] Yoshii, Y., and Hayasi, N., Sci. Rep. Tôhoku Univ., 4th ser., 1931, 6: 307.

[2] Iwata, E., Seitaigaku-Kenkyû, 1941, 7: 27.

with stunted growths of Abies Mariesii, which invade the bog coming in places close to the borders of the pools or in patches. The shores are projected lakeward. The bog surface is some 2 dm. higher than the pool's surface at the waterside, and the water depth there measures from 1 to 5 dm. in three pools other than Hyôtan-Numa, where it is deeper, viz. 3 to 6 dm.

All the basins are fed by seepage and filled with brown acid water, whose colours on the platinum-cobalt standard and hydrogen-ion concentrations are given in Table I, the transparency measured by means of the Secchi's disk being 2 m. in Naga-Numa It is interesting to note that the water of Hyôtan-Numa is much clearer than the others. It is noticed, on the other hand, that the former is at the highest point of this bog uneven in topography, Tsuki-Numa being on nearly the same level and Naga-Numa and Suiren-Numa on the lowest. These facts account for th. t Hyôtan-Numa is fed solely by the clear underground water seeping at its bottom, whereas in the other three the water supply comes from the overflow from Hyôtan-Numa, at the height, the water being coloured brown on coming through the peat layer, though they may be fed in part by their own seepage.

Temperature. No distinct thermal conditions can be noticed in any of these four. Thus measurements taken simultaneously of the surface waters may give a slight difference no greater than 2°. On the basis of observations from time to time during summer the maximum temperature of the surface water is presumably to be about 27° in midsummer. Not infrequently in that period, however, it may scarcely reach 20° in the early morning, even falling to 15° after prolonged rain. The water temperature at the deepest bottom of Naga-Numa is conjectured to lie at about 15° throughout summer. Some temperature gradient with a temporary thermocline at a depth less than 1 m. takes place on bright summer days. In winter it is bitterly cold and very snowy here, these pools being undoubtedly under ice then. The air temperature will sink even to some -20° in midwinter, and in the neighbourhood there lies snow invariably late into the summer.

Dissolved Oxygen. Dissolved oxygen was determined by Winkler's method, Alsterberg's[1] modification devised for water rich in organic matter, etc. having proved to give nearly the same value as was obtained by the original in this case. As will be seen from Table I, more permanganate consumption, and hence higher content of organic matter, are associated with lower oxygen content. Moreover the more the permanganate consumption, the deeper is the water colour. In the Menyanthes zone along the shallow shore, water is coloured more deeply with more organic matter than the open water, the permanganate consumption amounting to 45 to 66 mg. per l., but especially at sunny midday supersaturated, as high as 135%, with oxygen given out in the process of photosynthesis by littoral plants. The vertical distribution of dissolved oxygen determined one dull afternoon is as follows:—89% in the surface water, 77% at a depth of 1 m., and 48% in the bottom water—at a

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[1] Alsterberg, G., Biochem. Ztschr., 1926, 170 : 30.

depth of 2 m. Thus it decreases materially with depth. From the foregoing, it is clear that the organic matter partly distributed in the water and partly deposited at the bottom exerts a remarkable effect on the reduction in the concentration of oxygen. Actually the bottom mud is capable of absorbing oxygen vigorously as will be seen later (see Part II).

Hydrochemical Data.[1] In Table II are given the results of chemical analyses of the surface water which were carried out in early autumn according in the main to Maucha[2], the determination of carbonic acid in particular being carried out in the field about noon of a cloudy day. Of particular interest is the high content of sulphate ions. This suggests that the seepage contains sulphuric acid for which the volcanic activity is doubtless responsible. In reality, not far from here are hot springs where extremely acid water rich in sulphuric and hydrochloric acids flows. It is seen in Tab. I that pH is fixed at about 5.0 throughout the four pools irrespective of their different contents of organic matter, even the clearest one being as acid as those dark in colour. The effect of the organic acids on the pH is apparently nil. It appears that it does not come into question in the presence of the strong mineral acid, so that they do not enhance the acidity.

Table II. Analytical results of the surface water of Naga-Numa, in mg. per 1.

Suspended matter	Solids in solution	Loss on ignition of solids in solution	CO_2	HCO_3'	CO_3''	SiO_2	PO_4'''
5	25	15	4.7	4.9	0	2.0	0.05
SO_4''	Cl'	$Ca^{\cdot\cdot}$	$Fe^{\cdot\cdot}$	$Mn^{\cdot\cdot}$	$NH_4^{\cdot}$	NO_2'	NO_3'
19	1.7	1.0	0.15	trace	0.2	0	0

Acid Seepage in Relation to Bog Development. It seems likely that the acidity of the seepage itself has taken part in the development of the bog, being a deciding factor as to it concurrently with the cold climate. This reminds me of the conception of Miki[3], who, having worked with Mizorogaike on the outskirts of the City of Kyôto, a dystrophic pool receiving acid seepage, pointed out that most of bogs in Japan are bound up with volcanic regions and put forward the view that the acidity of seepage may be an important reason for their development.

Lake Type. It is clear that the pools in question are of a dystrophic type. Unlike the rest, however, Hyôtan-Numa is characterized by a lower content of humus and accordingly less typical. The only reason for it is, as stated above, that this pool, in particular, is fed exclusively by seepage on account of its high situation. It has already been recorded by Hada[4] that in Hokkaidô varied types of lakes with a startling contrast can be found in the same bog as will be mentioned later.

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[1] Yoshimura showed that the electric conductivity of the pool water was very low, as was expected for such dystrophic waters (See Yoshimura, S., Koshô-Gaku, 1937, p. 171).

[2] Maucha, R., Hydrochemische Methoden in der Limnologie, 1932.

[3] Miki, S., Kyôto-Fu Shiseki-Meishô-Tennenkinenbutsu Chôsa Hôkoku, 1929, 10: 61.

[4] Hada, Y., Nippon-Gakujutsu-Kyôkai Hôkoku, 1938, 13: 431.

AQUATIC VEGETATION[1]

Distribution. Leaving only a small bare area in the centre of Naga-Numa, there thrive water plants all over the bottoms of the four pools. As has already been noted by Horikawa[2] and Iwata, the whole aquatic vegetation may be divided into two zones. The shoreward zone is dominated by Menyanthes trifoliata, with which comparatively shallow shores are fringed. This extends from the waterside onward up to depths of from 3 to 4 dm. Adjoining to it, lies the second zone which is characterized by a water lily (Nymphaea japono-koreana) and covers most of the pool surface to a depth of about 1.5 m. (seldom reaching 2 m.). Since the depth at water's edge may often measure even 5 or 6 dm., that is, beyond the lower limit of growth of Menyanthes, the Menyanthes zone is not developed along the whole shore but confined to shallower parts.

The quantity and the vertical distribution of each hydrophyte are given in Table III. Speaking broadly, no remarkable variation from pool to pool cannot be recognized in these respects. Menyanthes and Nymphaea in particular are universally distributed throughout their own scopes of depth capable of growing, whereas the other plants grow occasionally in distinct patches.

Table III. Species of aquatics present, their quantities and vertical distribution.

Increasing quantities are indicated by increasing numbers, 1 to 5.
Figures in brackets refer to the limits of sparse growths.

Species	Quantity				Distribution in depth	
	Naga-Numa	Suiren-Numa	Hyôtan-Numa	Tsuki-Numa	Upper limit dm.	Lower limit dm.
Nymphaea japono-koreana	3	5	4	4	3(2)	15(20)
Menyanthes trifoliata	3	3	1	4	1	4
Utricularia tenuicaulis	4	4	0	4	1	17(20)
Sparganium hyperboreum	3	0	4	5	3(2)	15
Isoetes asiatica	3	2	3	2	3	13
Potamogeton Fryeri	4	3	0	0	5(2)	17(20)
Heleocharis mamillata	3	3	2	2	1	10
Scirpus Hotarui	2	2	0	1	1	3
Drepanocladus fluitans (a moss)	4	0	2	0	1	10
Phragmites vulgaris	3	2	1	1	1	5
Carex rhynchophysa	0	1	0	0	1	3

Batrachospermum is found in every pool, comparatively abundant in Hyôtun-Numa in particular.

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[1] I wish to acknowledge gratefully the assistance received from Dr M. Tatewaki and Dr S. Miki who kindly made the identification of the water plants.

[2] Horikawa, Y., Sci. Rep. Tôhoku Univ., 4th ser., 1930, 5: 555.

Process of Reclamation. By boring the bog at several points one or two metres apart from the edge of Naga-Numa, an old lacustrine deposit intercalated between the layer of older peat and the basal volcanic ash was discovered (see Part II). It is evident that reclamation has once occurred here. Actually every stage of reclamation is clearly displayed especially in this pool. It takes place through the following three steps: in the course of time, the Menyanthes zone becomes progressively shallower owing to superimposed development of the long, thick rhizomes of this plant, and this brings about, at last, colonization in it of Lobelia sessilifolia together with some comparatively large sedges, such as Carex limosa and C. Middendorfii. Presently, due to copious growths of these herbs knitting together, the water depth is diminished and approaches nil. Meanwhile Scheuchzeria palustris, too, appears sparsely. As a consequence, growth of Sphagnum sets in, whereby complete reclamation takes place ultimately, a Sphagnum-mat with Oxycoccus quadripetala and Drosera rotundifolia being formed.

Precise observation of the parts of the shores where there is no marked reclamation now proceeding, shows that they are, as a rule, fringed with a very narrow band of Sphagnum carpet with the same floral composition, but with an insignificant bredth, as compared with that just mentioned. There grow sparsely Menyanthes, Lobelia and the sedges attached to the waterside. Lysichiton camtschatense, Hosta longissima var. brevifolia and some others, too, are found on the border. This marginal mat merges into another community of plants covering relatively dry areas in the bog, where sedges are dominant and, in addition, grasses, Sanguisorba tenuifolia var. alba, Hosta longissima var. brevifolia, and so forth flourish.

The bog surface adjacent to the pools is getting higher and higher, because there is being deposited peat all the time. As a result of this, the water-level of the pools having no outlet is being raised. Needless to say, however, accumulation of the remains of aquatics on the bottom results in a decrease to some extent in the depth on the other hand.

Except where a broad zone of unstable Sphagnum-mat resulting from recent reclamation lies, the submerged side walls of the pools are of similar configulation. The upper major part of the side wall is nearly vertical while the lower minor part is much scooped. This interesting fact shows that the reclamation in the manner just mentioned has occurred in the past when the shore was shallow, and since then the shore practically remained, as it were, stationary, neither lakeward extension of the bog vegetation nor consequent further reduction of the pool surface taking place. Therefore, unless any extraordinary external change will set in for some reason or other, the present morphology of the pools, on the whole, will last long with the exception of the parts where the shore is still shallow.

Floating Island. Lobelia, associated with the sedges mentioned, shows a tendency to grow densely at the very lakeward margin of the Menyanthes zone preferentially, held, without rooted in the bottom mud, by the suspended tips of rhizomes of Menyanthes which are extended lakeward. This noteworthy habit of them leads to the initiation of reclamation from the outermost part of the Menyanthes zone, which frequently results in formation of a floating island. Also after a floating island begins to develop, the growth of Lobelia and the sedges is vigorous particularly at its outer edge, and thereby the island tends to enlarge lakeward, with the consequence that the depth of the water at the edge often reaches even 1 m. The floating island, separated from the shore by the Menyanthes zone, is liable to occur especially at the end of a headland, facilitated by its subaqueous topography. In Naga-Numa there are three well-developed floating islands. Being moored by the tough rhizomes of Menyanthes the floating islands here found are incapable of drifting about freely.

I shall consider the reason why Lobelia and the sedges do colonize preferentially the lakeward margin of the Menyanthes zone, avoiding the shallow interior of the zone. Lobelia may grow luxuriantly as well on water-logged peaty soil by the pools as in water. It seems most likely that, when Lobelia germinates in spring, the shallow Menyanthes zone is still covered with snow or receives cold thaw water coming from the surrounding bog and is liable to be too cold to admit of the germination, whereas the margin of the zone is facing to the open water which is to be warmed by sunshine. The soil temperature, too, is obviously then higher than in the water inside the Menyanthes zone.

Comparison with Other Bog Lakes. The bog lakes in question—Suiren-Numa—will be compared with upwards of one hundred pools in another bog called Kenashitai on this mountain, of which bog Prof. Yoshii has elucidated the general characteristics as stated above. The latter are small, even the largest and deepest one—Maru-Numa—having an area of 250 sq. m. and being nowhere more than 2 m. deep. Despite their properties similar to Suiren-Numa, as might be anticipated, let us say, a pH of about 5, non-occurrence is observed of some of the water plants grown abundantly in Suiren-Numa, that is, the water lily, Utricularia tenuicaulis, and the moss, Drepanocladus fluitans. Moreover, the scarcity of Lobelia and the consequent lack of floating islands are noticed. As in Suiren-Numa, it is not uncommon that a plant occurring in one pool lacks in another even very close to it. It is certain that this is not due to any marked variation in environmental conditions. The failure to grow is probably more related to the lack of opportunities for seeds, etc. to be conveyed from one pool to another.

Furthermore, some comparison will be made of these bog lakes of Mount Hakkôda with others found elsewhere in Japan. While in Hokkaidô bogs are distributed also in the lowlands, in the Main Island as well as in regions lying to the south they are usually restricted within mountains, among which Mount Hakkôda is particularly rich in bogs. The same can be said of dystrophic lakes which are intimately connected with bogs as a rule.

However, bog lakes are not necessarily dystrophic. Hada[1] classified, basing upon his extensive survey, bog lakes in

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[1] Hada, 1938, l. c.

Hokkaidô and Kurile Islands into four types, viz. olygo-, meso-, eu- and dystrophic. Of particular interest may be his so-called olygotrophic ones, which are represented by those in Sphagnum-bogs 1500 m. above sea-level on a volcano called Taisetsu-San[1]. They, generally 1.5 m. deep, are filled with acid water having a pH of 4.7 for the most part, but colourless with less permanganate consumption—2 to 11 mg. per 1. There is a deficiency of dissolved matter, which is said to render these waters less productive, supporting a poorly developed vegetation. It seems that in this case, too, the acidity is due to the mineral acid connected with the volcanic action. On the other hand, bog lakes with brown water but of a rather eutrophic nature are known in Hokkaidô and in Saghalien. They develop, as a rule, less acid reactions, the pH exceeding 6.

Most of typically dystrophic bog lakes hitherto recorded are in the mountainous centre of the country as well as Hokkaidô. Broadly speaking, they are less than a few ha. in area and no deeper than several metres and filled with water whose pH falls within the range 4.5 to 5.5 and whose permanganate consumption varies from 15 to more than 60 mg. per 1. Hydrophytes of very common occurrence in them are as follows: Brasenia purpurea, Nymphaea japono-koreana, Potamogeton Fryeri, Nuphar subpumilum, Nuphar subintegerrimum, Menyanthes trifoliata, Equisetum palustre, Heleocharis mamillata, Scirpus Hotarui, Scirpus Tabernaemontani, Phragmites longivalvis, Carex rhynchophysa, various species of Utricularia, Batrachospermum, Spirogyra. The majority of the plants above enumerated equally flourish in Suiren-Numa, and conversely many of those found in the latter are among those listed above. Hence the aquatic vegetation of Suiren-Numa is much the same as the other bog lakes in general and by no means specific.

Floating islands are known in Japan especially in shallow dystrophic lakes. As to the genesis of them, it has been described that they were formed in part because the projected shore broke loose due, for instance, to a change in the water-level and in part because of floating up of the layer of rhizomes of rooted water plants, torn up from the bottom owing mainly to gas evolution in the bottom mud in summer-time. The former refers only to the final stage which may eventually take place also in those like Suiren-Numa, but the steps involved in the course of development of a floating island, as stated above, have never been recorded before.

Compensation Point of Isoetes. Isoetes asiatica flourishing on the bottoms of the pools at depths ranging from 3 dm. to 1.3 m. is a small water plant whose height measures only several centimetres, and thereby provides a good material for such an experimentation as on the compensation point, which I conducted on a clear summer day towards the end of August in Naga-Numa.

After removed roots, an individual, weighed 0.8 to 1.9 g., was put in a bottle for oxygen-determination, which was filled with the surface water. A series of such bottles, inverted, were lowered to different depths with a distance of a half metre and kept submerged for 2¾

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[1] See Hada, Y., Seitaigaku-Kenkyû, 1939, 5: 267.

hours—from 12.45 to 15.30. Two bottles with the plant and two others without the plant—as the control—were set at each depth. In Table IV is set out the gain or loss in oxygen per gram of the fresh weight of the plant. It will be seen that the assimilation attains maximum at the surface, that is to say, in full light and the compensation point occurs at a depth of between 1.5 and 2 m. The compensation point lies far below the lower limit of distribution—a depth of 1.3 m.

Table IV. Gain or loss in oxygen per gram of Isoetes at varying depths.

Depth	Oxygen balance	
m.	c.c.	%
0	0.22	100
0.5	0.18	82
1.0	0.13	59
1.5	0.06	27
2.0	−0.01	−5
2.25	−0.01	−5

At the same time the penetration of light was measured by the use of the potassium iodide-sulphuric acid method[1]. It is, however, impossible to reveal the light factor in such a brown lake in this way without taking the red light into account. Because this method is sensitive almost exclusively to the blue light which is absorbed to a much greater degree than the red in the brown water, the latter penetrating to greater depths. 20 c.c. each of N/10 KI and N/2 H_2SO_4 were filled into large test-tubes, which were lowered to varying depths with an interval of ½ m. After a 3⅔ hours' exposure to light, from 10.05 to 13.45, the resulting liberation of iodine was determined by titration with N/1000 sodium thiosulphate. The latter solution required reached 54.3 c.c. at the surface. It fell to 5.0 c.c. at a depth of 0.5 m., in other words, light was reduced to 9% of full light. At a depth of 1 m. most of the blue light was cut off from the plant, but 0.3% being available; and, at last, at a depth of 1.5 m. it was practically dark, so far as the blue light is concerned. It was demonstrated in, say, Midge Lake[2], a brown lake in Wisconsin of the United States, that the blue light fell to 10, 1 and less than 0.1% of that at the surface at depths of 0.6, 1.2 and 2 m. respectively, whereas the red fell to 10, 1 and less than 0.1% at 1.8, 3.6 and 6 m., namely the red penetrated three times deeper than the blue. Again, in Rudolf Lake in that state, the blue light is almost unable to penetrate to depths over 2 m. and at a depth of 5 m. light consists, in fact, solely of the red and orange. Therefore, it is considered that also in Naga-Numa a considerable amount of long-waved rays penetrates much more deeply than the value just mentioned. The fact that the compensation point of Isoetes lies at a depth of more than 1.5 m. affords evidence for it.

NOTES ON ANIMAL LIFE

In conclusion brief mention will be made of animal life in Suiren-Numa, though the exhaustive treatment of it is impossible to the writer and is out-

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[1] See McCrea, R. H., J. Ecol., 1923, 11 : 103.

[2] See Clarke, G. L., Problems of Lake Biology, 1939, 27.

side the scope of this article.

From the mud on the deepest bottom of Naga-Numa a good many of Chironomus larvae were collected by means of the Ekman-Birge dredge. The chief members of the plankton in Naga-Numa are rotifers, cladocera and copepods, each of which occur in a great number. Thus Okada and Horasawa[1] found in these and other bog lakes in this mountain enormous numbers of Daphnia longispina, Chydorus sphaericus and Diaptomus pacificus, which were known to be plentiful in acid lakes in general. In addition to the zooplankton are frequently present desmids as well as diatoms whose tests are found in large numbers in the bottom mud. In these pools newts dwell and frogs spawn, while fishes are absent. A variety of water insects, too, pass their whole life-cycle or certain stages of their development therein.

SUMMARY

1. A group of bog lakes on Mount Hakkôda, an extinct volcano, are fed by acid seepage containing sulphuric acid (19 mg. SO_4'' per l.), which acid is considered to be linked with the volcanic action and, on the other hand, to be responsible for the development of the bog, concurrently with the cold climate there.

2. The pH value of the brown pool water is about 5.0; the permanganate consumption lies between 17 and 52 mg. per l. In the largest (2 a.) and deepest (2.5 m.) pool in particular, the bottom water is half-saturated with oxygen.

3. The shore is fringed with a zone of Menyanthes trifoliata, except where the depth at the water's edge is too great. From a depth of 3 or 4 dm. onward, adjoining to this, occurs a zone dominated by Nymphaea japonokoreana. In the centre of the largest pool an area deeper than 2 m. is left free from plants.

4. Rich colonization of Lobelia sessilifolia together with certain sedges in the Menyanthes zone, followed by development of a Sphagnum-mat, leads to reclamation. Lobelia tends to grow densely at the lakeward margin of the Menyanthes zone, resulting occasionally in formation of floating islands.

5. The compensation point of Isoetes asiatica was found to lie at a depth of between 1.5 and 2 m.

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[1] Okada, Y., and Horasawa, I., Seitaigaku-Kenkyû, 1935, 1: 265.

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PART II. BOTTOM DEPOSIT.

BY

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BOTTOM DEPOSIT[2]

Recent Bottom Deposit. The bottoms of the pools are everywhere covered with greyish black mud without any particular smell. In the middle of each of two basins, principal and accessory, of Naga-Numa, there is an area deeper than 2 m., where water plants fail to grow. Here the mud, measuring some 10 cm. in depth, is fine and soft and almost free from discernible plant residues, the only macroscopic inhabitants being Chironomus larvae. I have worked almost exclusively with this mud from the deepest plant-free bottom. The bottom mud of shallower parts covering itself with vegetation is penetrated by the roots and rhizomes of water plants and of more or less peaty texture due to admixture of the remains of aquatics once grown there, the false bottom being noticed in some shallow places.

Stratification. By boring the bottom, existence of a layer of volcanic ash underneath the mud was revealed. I used as a borer in this case a hollow piece of bamboo stem 5 cm. or so in diameter cut lengthwise into two halves, which, when used, were rejoined, bound with a string and fastened to a long bamboo pole. It was driven into the mud, being handled on a boat. The layer of volcanic ash is too hard to be pierced with such a simple bamboo tube, even an elaborate metallic tube sampler would yield no better result.

The inability to examine beneath has enforced me to rely upon an indirect way of boring the adjoining bog and drawing conclusions therefrom. Columnar samples taken from several spots close to the pools show four alternating layers of peat and volcanic ash in the bog profile. Thus, under the surface peat layer bearing the living vegetation, there lies a layer of volcanic ash, which in its turn overlies another layer of older peat, and again a basal layer of volcanic ash comes under the latter. Moreover, between the older

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[1] The writer repeats the acknowledgement made in the previous paper in this series to the Department of Education for a grant from the Scientific Research Expenditure.

[2] The mineralogical informations involved herein are due to the late Prof. K. Takane and to Dr K. Yagi, whom I desire to thank sincerely.

peat and the basal ash at some spots round Naga-Numa is noticed a well-defined layer of veritable old lacustrine sediment, which witnesses reclamation in the past. It seems very likely that this old bottom deposit extends to beneath the present lake bottom, lying under the upper ash layer and overlying the basal volcanic ash.

Of the two peat layers in the bog profile, the upper one is dark brown, while the lower is coloured almost black and is more compact in texture. The thickness of these peat layers differs among different places, varying from 30 to 60 dm. in the upper and 3 to 40 cm. in the lower. The layer of volcanic ash intercalated between the recent and old peat layers is 4 to 20 cm. thick and looks grey or greyish brown, whilst the volcanic ash at the base is tinged with a bluish hue. The former layer of volcanic ash is quite the same as that under the bottom mud in colour and texture. Microscopic examination as well shows that both are identical in all respects. There is no doubt whatever that these two are nothing but distinct portions of the same layer of volcanic ash.

Microscopic Findings. The volcanic ash underlying both the recent mud and the upper peat consists, beside a very small amount of minerals like crystals of feldspar, solely of fresh pointed fragments of pumice, which are transparent and colourless or with a brownish tint, no admixture of organic matter being observed. On the contrary, in the basal old volcanic ash the pumice fragments have been considerably decomposed and have changed opaque and lost sharpness, feldspar also decomposed to some extent being only seldom found.

The major part of the mud covering the deepest plant-free bottom, whose loss on ignition amounts to 33%, is made up of mineral matter. It consists of the fresh hyaline fragments of pumice of just the same nature as that in the upper layer of volcanic ash. It is indubitable that it is derived from the latter, coming far from outside the pool and, after once suspended in the water, sooner or later deposited at the bottom together with organic matter. The mud contains numerous diatomaceous tests, pollen grains and other plant residues. The fossil diatoms are dominated by Melosira.

The old bottom deposit beneath is very similar to the recent deposit with respect to the organic constituents, involving diatomaceous tests, likewise chiefly of Melosira, as well as vegetable remains including pollen grains. But, in contrast to this, they are quite distinct in the mineral constituents. Whilst in the latter, as has been mentioned already, the pumice fragments are fresh and hyaline, those in the former have been decomposed to a large degree and consequently have lost the sharp edges and look opaque. It is thus clear that the mineral particles in the old bottom deposit are undoubtedly derived from the basal volcanic ash.

Oxygen-Absorbing Capacity. Lönnerblad[1], working with Swedish lakes, demonstrated that the bottom muds of dystrophic waters took up a large quantity of oxygen when put into common water. When 20 g. mud was put in a stoppered 150-c.c. bottle filled up with water rich in oxygen, the oxygen dis-

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[1] Lönnerblad, G., Bot. Notiser, 1930, 53.

appeared entirely from the water in 12 to 18 hours in general, at times even in 6 and odd hours. And in this case previous sterilization exerted no remarkable influence upon it. However, those of eutrophic lakes which also displayed so strong an oxygen uptake as those of dystrophic ones lost most of the capacity on sterilization. On the basis of these experimental data he advanced a view that the power of strongly absorbing oxygen widespread throughout the dystrophic muds is to be ascribed principally to the purely chemical reducing power of humus, without the intervention of micro-organisms, whilst that of the eutrophic muds depends largely upon the activity of microbes existing therein.

Lönnerblad's experiments were repeated using the mud from the deepest bare bottom of Naga-Numa, and his findings proved to hold for our material. Mud taken by means of the Ekman-Birge dredge was put in lots of 30 g. in batches of 250-c.c bottles, to which was added up to the top tap water saturated with oxygen. After stoppered without leaving bubbles and shaken enough, the bottles stood at room temperature (some 15 to 20°). The supernatant water in each of the bottles was poured into a bottle for oxygen-determination and the oxygen left was estimated by Winkler's method at the end of different periods, --for 3, 9 and 23 hours. Besides, a series of bottles without mud were set as the control. The three cases are illustrated in Table I. It is there seen that 30 g. of mud absorbed some 1 c.c. of oxygen for 24 hours, this amount corresponding to more than a half of oxygen dissolved in the tap water in the bottle. Moreover, on shortening the period of incubation to 10 minutes, it was brought out that about 0.7 c.c. of oxygen had already disappeared even in this short time. This points to the fact that quick removal of much of the oxygen took place at the very moment of contact. Admixture of 0.2 g. of corrosive sublimate had no effect. It is evident, therefore, that the oxygen consumption was not in virtue of microbes.

Table I. The progress of oxygen absorption by mud.

Bottle No.	Period of incubation hr.	Oxygen absorbed c. c.	
1	3	0.84	0.855
2		0.87	
3	9	0.81	0.82
4		0.83	
5	23	1.00	1.005
6		1.01	

As has already been mentioned, the water of the pools contains a brown substance—so-called humus—which causes the brown coloration of the water, and is capable of consuming permanganate, and, furthermore, is responsible for the diminution in the amount of oxygen in the water. There can be little doubt that the capacity of the mud to absorb oxygen so vigorously is due to the humus incorporated with it.

POLLEN-ANALYSES OF LACUSTRINE SEDIMENTS

That the lacustrine sediment provides an excellent material for the pollen-analysis comparable with the peat of bogs has been shown by a number of workers, notably Lundqvist[1], Groschopf[2], etc. Since some of the bogs on Mount Hakkôda have already been worked upon pollen-analytically first by myself[3] and afterwards by Nakamura[4] following up my work to a greater extent, an attempt to analyse for pollen the bottom deposits of the pools in question might be of interest.

Columnar samples of the recent bottom deposit taken with the bamboo tube from the deepest spot of Naga-Numa, on the one hand, and those of the bog profile adjacent to the pool including the intercalated old lacustrine deposit, on the other, were subjected to pollen-analyses.

It has been mentioned already that pollen grains incorporated into the recent and old bottom muds are very abundant. The most important ones among them are those of Abies, Pinus, Fagus, Quercus, Betula, Alnus, and Pterocarya, those of Ulmus being found in comparatively small amounts. Of course pollen grains of other kinds of woody and herbaceous plants are also present, yet insignificant in the quantity, too. In view of the woody species thriving actually in the neighbourhood, the above genera are conceivably to be represented by the following: Abies Mariesii, Pinus pumila, Fagus crenata, Quercus crispula, Betula Ermani var. communis, Alnus Maximowiczii, Alnus pendula, Pterocarya rhoifolia, Ulmus laciniata.

Here quotation in outline will be made of the woody vegetation of this mountain from papers of Yoshioka[5]. The highest part from a height of 1400 m. onward is covered with dwarf pines as well as alpine shrubs, such as alders. Next to this down to 900 m. come coniferous forests dominated by Abies Mariesii, which are, in turn, adjoined beneath by deciduous forests whose dominant tree is Fagus crenata. The upper part of the coniferous forest zone is occupied by pure fir woods, while the lower part is mixed with birches, some beech trees coming up to some extent. In the deciduous forests, associated with the beech trees, occur oaks, pterocaryas, elms, etc. Yoshioka regards the fir woods without or with admixture of birches or beeches and the pure beech woods as the climatic climaxes.

Suiren-Numa do lie at a height of 980 m., and therefore amid the zone of mixed forest of the fir and the beech. And in the very bushes immediately round them stunted growths of the fir are prominent mingling among a variety of trees and shrubs, among which are the dwarf pine, beech, oak, alder, and so on.[6]

The procedure employed is the same as described in our earlier papers for the peat. 200 grains of the above eight genera were counted and therefrom the percentages were calculated. Identification of pollen grains was carried out basing upon the atlases, which had, in part, been prepared by me[7] and subsequently supplemented and completed

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1) Lundqvist, G., Abderhaldens Handb. biol. Arbeitsmeth., 1925, Abt. 9, Teil 2, 427; Bodenablagerungen und Entwicklungstypen der Seen, 1927.
2) Groschopf, P., Arch. Hydrobiol., 1936, 30: 1.
3) Jimbô, T., Sci. Rep. Tôhoku Univ., 4th ser., 1932, 7: 129.
4) Nakamura, J., Seitaigaku-Kenkyû, 1942, 8: 18.
5) Yoshioka, K., Seitaigaku-Kenkyû, 1937/1938, 3: 187, 322, 4: 27, 150, 227, 352; 1943, 9: 187.
6) See Iwata, 1941, l. c.
7) Jimbô, T., Sci. Rep. Tôhoku Univ., 4th ser., 1933, 8: 287.

Table II. Pollen-analytical results of the recent lacustrine sediment, given in percentages.

Average of three columnar samples. The rows between the top and base represent levels with the same interval. This holds for the next table, too.

	Abies	Pinus	Fagus	Quercus	Betula	Alnus	Pterocarya	Ulmus
Top	10	16	31	15	15	7	3	2
	14	11	32	17	17	5	3	1
	10	7	40	14	14	8	5	2
Base	9	5	47	13	13	5	7	1

Table III. Pollen-analytical results of the bog profile, expressed by percentages.

Average of two columnar samples. Figures in brackets indicate values calculated assuming Pinus to be zero.

	Abies	Pinus	Fagus	Quercus	Betula	Alnus	Pterocarya	Ulmus
Upper peat layer:								
Top	9 (15)	43 (0)	18 (30)	12 (21)	11 (20)	4 (7)	4 (6)	1 (2)
	9	3	44	10	13	11	8	2
	12	5	51	11	10	6	6	1
	10	5	34	22	11	9	7	2
	10	1	43	8	8	22	6	2
Base	8	3	55	12	7	9	6	2
Lower peat layer:								
Top	10	3	50	9	12	5	9	3
	5	5	54	9	9	6	11	4
	8	5	35	14	12	12	12	2
Base	12	3	49	13	8	7	8	2
Old lacustrine sediment	4	2	47	14	15	7	10	1

by Nakamura.[1]

The results obtained are given in Table II and III.

Comparison between the recent bottom deposit and the upper peat layer—both being contemporary—draws at once attention to the peculiar fact that in the upper peat layer, unlike in the bottom mud, pollen of Pinus is concentrated at the very surface. A value as high as 43% is recorded for pine pollen at the uppermost level of the upper peat layer, whereas it falls to only 1 to 5% not only throughout the rest but also in the lower

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[1] Nakamura, J., Sci. Rep. Tôhoku Univ., 4th ser., 1943, 17: 491.

peat layer. It appears to be responsible for it that in virtue of the presence of air sacs and the smallness pine pollen is liable to float on the surface of water permeating the bog. That sample was taken actually from the very surface and was nothing but a rough mass of plant remains still retaining the original forms. In the pool, on the contrary, such pollen grains will float on the water surface and by no means be capable of lying upon the bottom to give such a high value as at the surface of the peat. It is noted that, on the whole, the percentage of pine pollen is somewhat higher in the mud than in the peat. This fact leads to the conclusion that, pine pollen which had fallen on the bog surface and was floating on the surface of the permeating water was partly washed into the pool by rain and so forth, and subsequently sank to the bottom together with what had fallen directly on the pool surface . Contrary to all expectation such a fact cannot at all be observed in pollen of Abies in spite of the marked similarity to that of Pinus in every respect but the much larger size. It is thought, at all events, not to float so readily as pine pollen.

In the bottom deposit, Fagus increases with increasing depth, while Pinus decreases. Furthermore, a tendency toward a decrease in Abis and an increase in Pterocarya may be recognized, but Quercus, Betula, Alnus and Ulmus remain nearly constant.

The values of the surface layer of peat are, as we have seen, materially distorted by the floating Pinus pollen, so that it would be appropriate to consider the values obtained by assuming Pinus to be zero. In the upper peat layer the marked fluctuation as is characteristic of some trees in the mud is not so manifest. But no results entirely conflicting with those from the mud were obtained, except for the case of Pinus mentioned above. Similar to the upper peat layer, in the main, is the lower peat layer, and the old lacustrine sediment underlying it as well.

Working with the bog of Kenashitai already referred to, I have previously brought out that Fagus increased and Abies simultaneously fell off with the depth remarkably. Generally Pinus was less abundant at lower levels, whilst Betula and Alnus did not behave in any noticeable way. Later Nakamura worked on three other bogs. Results obtained by him corroborated mine on the whole, and the fact that Fagus was more abundant and, on the contrary, Abies and Pinus more scarce on the levels nearer the surface, was much emphasized. Betula, Alnus, Pterocarya and Quercus were almost invariable throughout. These findings led to the conclusion that in older times flourished deciduous trees, whose upper limit was higher than at present, but they afterwards gave way to conifers, which came down invading the deciduous forests. It seems to follow from this that there was a concomitant change in the climate getting colder and colder as time went on.

The above points are not so pronounced in the results obtained in the present investigation regarding Suiren-Numa and the surrounding bog, especially in the peat, whilst they are seen more or less clearly in the lacustrine sediment. Why do the lake deposit and the peat of the bog round the pool not give the same results? Although at present I am unable to account for it, it should be noted that the circumstances under which pollen grains were deposited were by no means identical. It is exemplified in the fact described above that the distortion resulting from the floating habit of pine pollen is seen in the peat alone. At least in this respect the lake deposit is doubtless more suitable for the pollen analysis than the peat.

SUMMARY

1. Blackish, soft mud covers the deepest plant-free bottom. It is capable of taking up a large quantity of oxygen, as Lönnerblad has recorded in Swedish bog lakes.

2. The bottom mud overlies a layer of volcanic ash. It is very likely that, though the volcanic ash precludes sampling beneath, there lies under it an old bottom deposit, which is actually found in the bog profile close to the water's edge.

3. The bottom deposits, rich in fossil pollen, proved to be an excellent material for pollen-analysis, reflecting the descending of conifers invading deciduous woods in the past, a fact previously brought out pollen-analytically by us working with the peats of bogs in this mountain. It was noticed, however, that, between the lacustrine sediment and the peat of bogs, there exists some difference in the circumstances under which pollen grains have been deposited, with the consequence that both the materials do not necessarily give the same result.

In conclusion, I wish to express my best thanks to Prof. Y. Yoshii for the facilities he has offered me for carrying out this investigation.

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ARTIFICIAL ALTERATION OF THE EMBRYONIC AXIS IN THE CENTRIFUGED EGGS OF SEA URCHINS.

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It has been found that in many forms the position of the embryonic axis is not affected by changing experimentally the distribution of the endoplasmic materials in the egg cytoplasm. The centrifuged eggs developed normally with the direction of the embryonic axis independent to that of the centrifugal force. In 1933 and 1935, the present writer pointed out on the basis of morphological observation that the cortical cytoplasm of sea urchins' eggs showed no change in its structure before and after centrifuging, and accordingly that the function of the cortical cytoplasm is preserved from the effect of the centrifugal force. Thus, it is suggested that the polarity of the egg is determined in the cortical cytoplasm.

Another evidence for this hypothesis is the behavior of the cortical cytoplasm in the developing egg of sea urchins. That is, in the course of the cleavage of the egg the cell boundary between the blastomeres is newly formed, and as the result, the cortical cytoplasm is not carried into the inner part of the embryo, but it remains always on the superficial position of the embryo.

It is evident that for positive proof of the importance of the cortical cytoplasm on the determination of the polarity of the egg it is necessary to show the possibility of artificial alteration of the polarity of the egg by affecting the cortical cytoplasm. In the present paper it is presented the results obtained from ultra-centrifuging of the eggs of the sea urchins, Strongylocentrotus pulcherrimus and Temnopleurus hardwickii, and to note the cases of the artificial alteration of their embryonic axis.

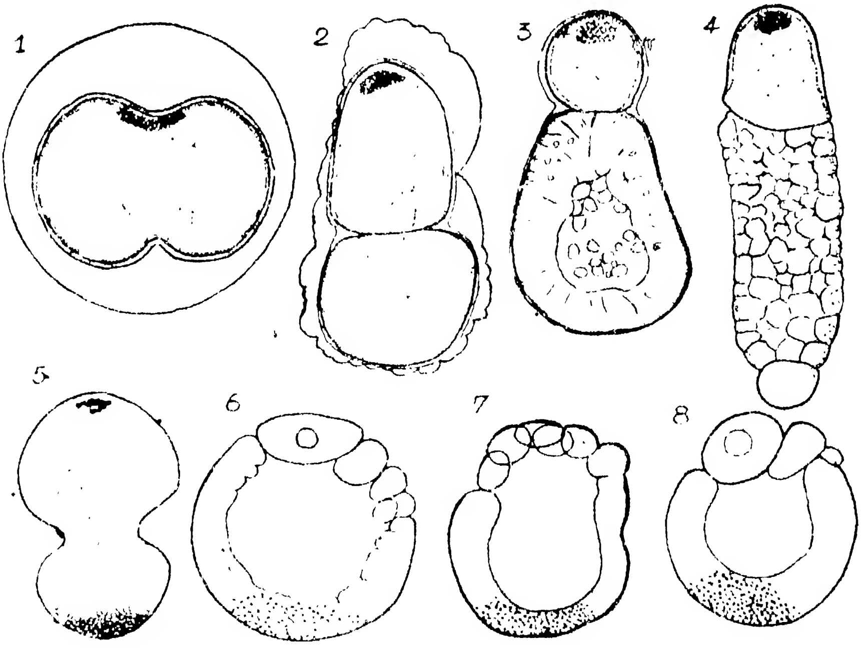
For centrifuging the eggs, an ultra-centrifuger of the air turbin type was used. The average number of revolutions per minutes was 40000, and its centrifugal force measured 40000 times gravity. As the unit of the force the product of gravity and time, gravity-minute (GM), was used.

I. CENTRIFUGING OF THE UNFERTILIZED EGGS.

The living egg of Strongylocentrotus pulcherrimus contains light orange pigment granules. They are observed in the cortical cytoplasm, but not in the endoplasm (Motomura 1935). When the unfertilized eggs were centrifuged with the force of $1.0 - 1.5 \times 10^5$ GM, the stratification of the endoplasm in 4 layers was observed; they are, 1) oil drop layer at the centripetal end, 2) clear cytoplasmic layer including the pronucleus, 3) yolk layer, and 4) clear layer at the centrifugal end. On the other hand, the cortical cytoplasm, which contains the pigment granules, remained unchanged its normal position. The janus green granules, which are also one of the structural elements of the cortical cytoplasm (Motomura 1936, 1941), were not shifted.

With the force of 5×10^5 GM, the eggs were elongated, and some of them were torn into two pieces of the centripetal and centrifugal halves. In this case the centripetal half contains a large quantity of the pigment granules, and this shows that the cortical cytoplasm were moved to the centripetal side. The pigment-containing layer, the cortical cytoplasm, was found close to the oil drop layer. The janus green granules were also shifted to the centripetal half.

The cleavage pattern of the centrifuged eggs were changed according to the intensity of the centrifugal force. When the unfertilized eggs were centrifuged with the force of 1×10^5 GM, they rounded up soon after fertilization, and were divided with the first



Figs. 1–8. Development of the centrifuged eggs; 1–4, eggs of Strongylocentrotus pulcherrimus, centrifuged before fertilization; 1, first cleavage centrifuged with 1×10^5 GM; 2, with 5×10^5 GM; 5–8, eggs of Temnopleurus hardwickii; 5, beginning of the first cleavage centrifuging the unfertilized egg with 5×10^5 GM; 6–8, blastula stage centrifuged shortly after fertilization with 5×10^5 GM.

cleavage plane perpendicular to the stratified layers (Fig. 1). They developed normally and became plutei. When the eggs were centrifuged with strong forces stronger than 5×10^5 GM, on the contrary, they were divided with the first cleavage parallel with the stratification (Figs. 2, 5). And the cleavage went on rapidly in the centrifugal half, whereas in the centripetal end it was distinctly inhibited, or stopped (Figs. 3, 4). As a result, a large yellow cell with a nucleus and oil drops was observed in the strongly centrifuged cases. The eggs did not develop the normal larvae, but they stopped at the blastula stage, when the cilia developed only on the surface of the small cells of the centrifugal side. The centripetal large yellow cells could not develop the cilia.

Those results show that with a strong centrifugal force the cleavage pattern as well as the morphological differentiation of the egg can be modified, and that the distortion depends mainly upon the change of distribution of the cortical cytoplasm. But as mentioned above, the eggs are elongated by the strong centrifuging, if the unfertilized eggs are centrifuged. They do not recover their round form. And, in many cases they are torn into pieces, because they are not enclosed in an envelope, such as the fertilization membrane.

II. CENTRIFUGING OF THE FERTILIZED EGGS.

As mentioned above, the unfertilized eggs are elongated and broken into pieces with a strong centrifugal force. This makes it difficult to see the relation between the centrifuging axis and the polarity of the egg. In the following experiments, therefore, the eggs were centrifuged after the formation of the normal fertilization membrane. In the fertilized eggs of Strongylocentrotus pulcherrimus the stratification of the endoplasm appeared with the force of 3×10^5 GM. But, the yellow layer, the layer of the cortical cytoplasm, appeared with the forces over 5×10^5 GM. In the former case the cleavage plane was perpendicular to the stratification, but in the latter it became parallel, and as a result, the giant cell was formed at the centripetal side (Figs. 10, 11).

In the eggs of Temnopleurus hardwickii the centripetal giant cell was formed, if the eggs were centrifuged with the force of $5 - 8 \times 10^5$ GM. (Figs. 6, 7, 8). In the centrifuged living egg of this species 4 layers are distinguished; they are, 1) oil drop layer at the centripetal end, 2) clear cytoplasmic layer with nuclei, 3) translucent granular layer, and 4) brown pigment layer at the centrifugal end. The pigment granules could be moved even with a weak centrifugal force, and consequently, it is in the endoplasm. The manner of the effect of the centrifugal force on the cleavage pattern was the same as in St. pulcherrimus. In a weak centrifuging stratification of the endoplasm begin to appear, the first cleavage plane moved to become perpendicular to the stratification. And, with the forces over 5×10^5 GM it showed the tendency to go parallel (Table 1).

In short, the formation of the giant cells was observed also by centrifuging the fertilized eggs, and the results were the same in the mentioned two species of sea urchins.

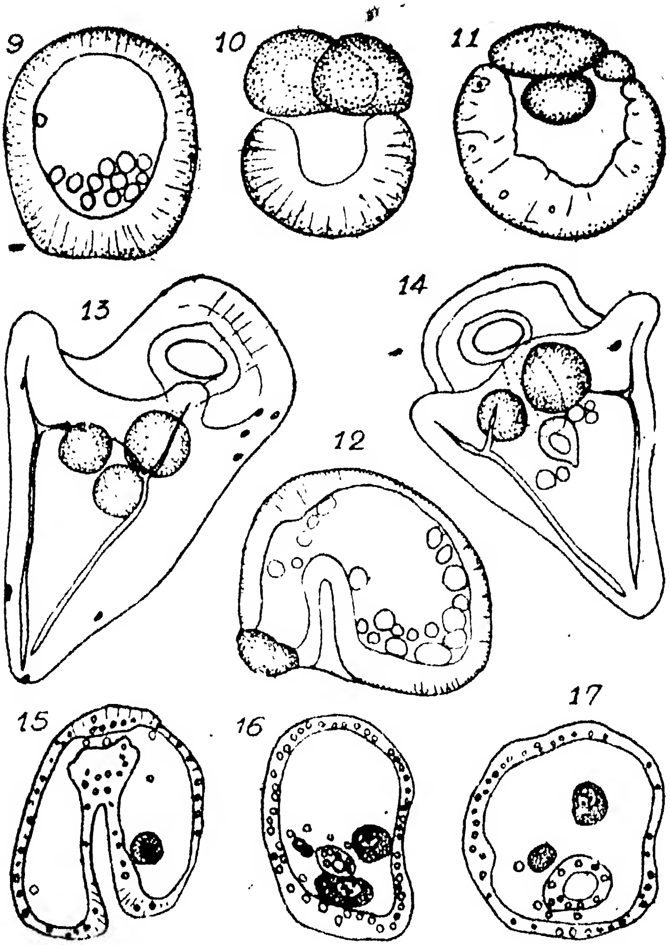
Table 1. Alteration of the first cleavage plane by centrifuging the fertilized eggs of Temnopleurus hardwickii.

Centrifugal force in GM.	Direction of the first cleavage plane in relation to the centrifugal force				Total
	Parallel	Oblique	Perpendicular	Indistinct	
2×10^5	59	43	2	6	111
4 〃	57	56	1	17	131
5 〃	30	56	56	4	146
8 〃	55	45	33	4	137

III. ALTERATION OF THE EMBRYONIC AXIS BY CENTRIFUGING.

A) Strongylocentrotus pulcherrimus. The embryonic axis is not influenced by a weak centrifuging. The present writer's results agreed with those reported by authorities. With a strong centrifugal force, on the other hand, a remarkable tendency of influence on the direction of the embryonic axis was observed. When the fertilized egg was centrifuged with the force of 8×10^5 GM a giant cell was formed at the centripetal end (Figs. 10, 11). The egg developed to the early gastrula stage. Nearly in all cases the giant cell was found in the ectoderm at the ventral side of the blastopore of the early gastrula (Fig. 12). The result agreed with that in Dendraster reported by Pease (1939). But, the effect was so heavy that the egg did not develop pluteus.

With the moderate centrifuging the egg developed to the pluteus, and its embryonic axis showed a remarkable relation with the direction of the centrifugal force, the centrifuging axis. Namely, when the fertilized eggs were centrifuged with the force of 5×10^5 — 7×10^5 GM, several giant cells with yellow pigment were formed at the centripetal end. They were not large in comparison with the former case, but were easily distinguished from the cells of the remaining portion. At the gastrula stage, the giant cells entered into the blastocoel in most cases, or they were found in the archenteron in a few cases (Figs. 15, 16, 17). At the early pluteus stage the centripetal oil drop or the yellow giant cells were found in the mesenchym or in the archenteron (Figs. 13, 14). Sometimes, a pluteus without archenteron was observed, but in this case the skeleton and two or three giant cells were found in the blastocoel (Fig. 13). From those facts it is concluded that the centripetal end becomes the vegetal pole of the moderately centrifuged egg (Table 2). According to the writer's opinion, Pease's cases in Dendraster agree with the strongly centrifuged cases of this paper, in which the normal process of gastrulation was disturbed by a centripetal giant cell.



Figs. 9—17. Strongylocentrotus pulcherrimus; 9, normal blastula; 10, 11, blastula stage centrifuged shortly after fertilization with 7×10⁵ GM, giant cells were found at the centripetal side; 12, gastrula of Pease's type, giant cell was found at the ventral side of the ectoderm, 8×10⁵ GM; 13, 14, pluteus of centrifuged eggs, giant cells were found in the blastocoel, 7×10⁵ GM; 15—17, sections of gastrula of centrifuged eggs, showing the giant cells in the blastocoel, 7×10⁵ GM.

Table 2. Position of the centripetal oil drop layer at the early pluteus stage of the eggs shortly after fertilization.

(Strongylocentrotus pulcherrimus)

	Animal hemisphere			Vegetal hemisphere		Indistinct	Total
	Apex	Side	Base	Endoderm	Mesoderm		
Number of eggs	4	10	16	76	262	69	437
Total	30			338		69	437

B) Temnopleurus hardwickii.

In the eggs of Temnopleurus the centrifugal pigment layer was taken as the mark of the centrifuging axis, because the oil drop layer of the centripetal end disappeared at the gastrula stage. When the fertilized eggs were centrifuged with the force of 4×10^5 GM, the pigment layer was found in the ectoderm as well as in the mesoderm and endoderm by chance (Table 3). In the case centrifuged with the force of 5×10^5 GM, the pigment layer was found in the ectoderm in 73% of the total eggs. And, when the fertilized eggs were centrifuged with 8×10^5 GM, nearly 90% of the eggs carried the pigment in the ectoderm. The centripetal giant cells were also found in the mesoderm as well as in the endoderm. The last mentioned two cases show remarkably the tendency of coincidence of the embryonic axis with the direction of the centrifugal force. And, in Temnopleurus, as well as in St. pulcherrimus, the centripetal pole became the vegetal pole, if the fertilized eggs were centrifuged with a force of a moderate intensity, with which the cortical cytoplasm could be moved to the centripetal side.

Table 3. Position of the centrifugal pigment layer at the early pluteus stage of the eggs centrifuged shortly after fertilization.
(Temnopleurus hardwickii)

Centrifugal force in GM.		Animal hemisphere			Vegetal hemisphere		Indistinct	Total
		Apex	Side	Base	Endoderm	Mesoderm		
4×10^6	Number of eggs	35	80	62	88	52		
	Total	117			140		30	347
5×10^5	Number of eggs	113	150	75	72	27		
	Total	356			100		30	486
8×10^6	Number of eggs	140	132	85	27	9		
	Total	357			36		19	412

IV. DISCUSSION.

In many species of sea urchins it has been shown that the unfertilized egg is torn or deformed with a strong centrifugal force. According to Harvey (1939) the egg of Arbacia is torn with a force of 4×10^4 to 8×10^4 GM. This was one of the difficulties for the study of the effects of centrifugal force on the embryonic axis. In the present paper, therefore, the effect of deformation and tearing was avoided by centrifuging the fertilized egg.

The results of centrifuging of the fertilized eggs were the same in Strongylocentrotus pulcherrimus, and Temnopleurus hardwickii. When the centrifugal force was from 2×10^5 to 4×10^5 GM, the first cleavage plane went perpendicular to the stratification, and the embryonic axis developed independently to the direction of the centrifugal force. With a centrifugal force over 5×10^5 GM, the first cleavage plane became parallel with the stratification, and the embryonic axis was strongly influenced in a way, that the embryonic axis coincides with the direction of the centrifugal force, and that the centripetal end becomes the vegetal pole.

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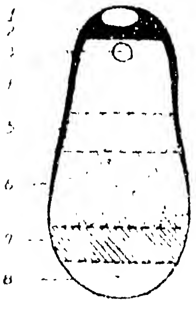

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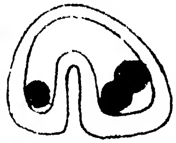

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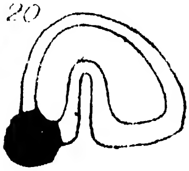

Fig. 18. Scheme of centrifuged unfertilized egg of St. pulcherrimus, 1) centripetal oil drop. 2) layer of cortical cytoplasm, 3) pronucleus, 4) hyaline cytoplasm, 5, 6, 7) yolk layer, 5) layer stained with brilliant cresyl blue, 6) layer stained with nile blue, 7) layer stained with neutral red, 8) centrifugal hyaline layer.

Fig. 19. Scheme of gastrula showing the position of the giant cells in the moderately centrifuged egg.

Fig. 20. The same in the strongly centrifuged egg, Pease's type.

Pease (1939) centrifuged the unfertilized egg of Dendraster with forces of 45,000 to 50,000 G for 6 to 8 minutes, that is about 270,000 to 400,000 GM, and he observed the formation of the centripetal lobe. According to him the lobe was found at the ventral region of the ectodermal tissue of gastrula in 93% of the total number of eggs. The present writer, also, observed the same type of deformation in the eggs centrifuged beyond the optimum intensity (Fig. 12). And, this was one of the extreme cases of the effect of the centrifugal force. The normal process of gastrulation was inhibited by the centripetal giant cell. According to the present writer's opinion, the primary influence of the centrifugal force on the embryonic axis was shown only in the case, in which the invagination of the endoderm was not inhibited by too large a centripetal cell.

Runnström (1926), Lindahl (1932) and Pease (1929) agreed in the points, that the stratification has no influence on the polarity axis determined by the animal and the vegetal poles, and that it changes the direction of the dorsoventral axis. The results reported in this paper do not agree with them.

The way in which the alteration of the embryonic axis takes place in the centrifuged eggs will be discussed here. In St. pulcherrimus the cortical cytoplasm can be marked by its own yellow pigment, which can easily be observed in blue light (Motomura 1935). In the strongly centrifuged unfertilized as well as fertilized eggs the pigment layer is formed close next to the oil drop layer, and it adjoins the superficial cortical cytoplasm of the middle as well as the centrifugal portion of the egg. As the result, the cortical cytoplasm of the centripetal side is thick in comparison with the centrifugal side. And, the phenomena of invagination of the centripetal side will easily be understood, if we assume that the invagination begins at the point where the cortical cytoplasm has a sufficient thickness. The model experiments of invagination carried out by authorities (Spek 1918, Lewis 1947 and others) showed that the mechanism can be interpreted with the difference of the colloidal nature between the outer and the inner sides of the cell layer. And, it has been found that the cortical cytoplasm is always at the superficial position till the gastrulation begin (Motomura 1935).

In 1935 the writer reported the cases of artificial formation of double tailed monsters in the centrifuged amphibian eggs. This is caused by forming the secondary invagination at the animal pole. The mechanism of the secondary invagination can be explained with the same hypothesis. The detailed account of this phenomenon will be given in the next paper.

SUMMARY

The effect of centrifugal force on the determination of the embryonic axis was tested by centrifuging the fertilized eggs of the sea urchins, Strongylocentrotus pulcherrimus and Temnopleurus hardwickii.

The cortical cytoplasm of the fertilized eggs was moved with the centrifugal force of over 5×10^5 gravity-minutes (GM).

The cleavage pattern as well as the direction of the embryonic axis were altered in accordance with the centripetal shifting of the cortical cytoplasm.

The centripetal pole became the vegetal pole of the centrifuged eggs.

The invagination began at the point where the cortical cytoplasm was shifted to a sufficient thickness.

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DOUBLE EMBRYOS CAUSED BY TWO DEVELOPING CENTRES IN CENTRIFUGED EGGS OF A TOAD.

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(With 16 Text-figures)

(Received September 30, 1948)

Twins may be produced from the frog's egg by inverting it in the two cell stage (Schultze 1894, Wetzel 1895). In this case the twinning is shown to be due to the rotation of the interior of the two blastomeres in such a way that the direction of invagination from the predetermined grey crescent are separated (Penners and Schleip 1928). In triton, twins can be obtained by constricting the egg at the two cell stage, or even later, in such a way that the region of gastrulation is divided into halves (Spemann 1914, 1928). In both of these cases the significant feature is that the position of the predetermined developing centre at the dorsal lip could not be changed by the experimental conditions.

In 1935, the writer reported the cases of artificial formation of double tailed monsters in the centrifuged eggs of a toad, Bufo vulgaris formosus Boulenger (Motomura 1935). This is caused by forming the secondary invagination at the animal pole. The region of the secondary invagination acquired newly the capacity of the organization centre, and as the result, the neural tube and tail were induced. In the preceding paper (Motomura 1948) it was reported that the mechanism of the alteration of the embryonic axis of sea urchins' eggs by ultra-centrifuging can be explained by assuming the thickness of cortical cytoplasm of the egg. And it is the purpose of this paper to contribute to a better understanding of the role of the cortical cytoplasm on the determination of the embryonic axis by giving a detailed description of the mode of production of the double tailed monsters.

The material used was the egg of a toad, Bufo vulgaris formosus Boulenger. For centrifuging an electric centrifuger was used. The numbers of revolutions per minutes used were 1000 to 2000. The centrifugal force varied according to the distance of radius and to the length of time applied. The forces, calculating with the product of gravity and time, measured from 450 to 3200 gravity-minutes (GM). The fertilized eggs were centrifuged before the beginning of the first cleavage without removing the jelly.

I. THE RATE OF APPEARANCE OF THE DOUBLE TAILED EMBRYOS BY CENTRIFUGING

Nearly 20,000 eggs were centrifuged with the forces from 450 to 3200 GM. Although the susceptibility of the eggs were different according to the batch of eggs and to the time after fertilization, the double embryos were usually formed when the centrifugal force reached nearly the limit of tolerance of the egg, and therefore, the disintegration of the eggs occurred with a slightly stronger force than the optimum for the double formation. In other words, the eggs of the centrifugal end of a centrifuging tube died, while those of the centripetal end developed normally. And, only a few eggs neighbouring the disintegrated ones developed the double tailed embryos.

An example to show the relation between the rate of double-tailed embryos and the intensity of the centrifugal force is given in Table 1. In this batch of eggs the double-tailed embryos were formed with the centrifugal force from 1200 to 3200 GM. But the rate of mortality increased with the intensity of the forces. The optimal intensity measured 2000 to 2400 GM. In the control, using a portion of the same batch, no double-tailed embryo was observed.

Table 1. Rate of the double-tailed embryos in centrifuged eggs.

Intensity of centrifugal force in GM	Number of double-tailed embryos	Number of single-tailed embryos including other deformations	Eggs died	Total	Per cent of double-tailed embryo for total living eggs
1200—1600	9	443	293	745	2.0
1600—2000	24	170	557	751	12.4
2000—2400	59	56	672	787	51.3
2400—2800	21	26	719	766	44.7
2800—3200	10	8	528	546	55.5

II. CLEAVAGE AND GASTRULATION OF THE CENTRIFUGED EGGS

When the fertilized eggs were centrifuged strongly, the white lipoid accumulated at the upper pole, the animal pole, and as the result, the black pigment was driven away from the pole. The cleavage did not proceed, because the egg cytolysed beginning from the upper pole. When the eggs were centrifuged weakly, they developed normally.

By centrifugal action of a moderate intensity, the black pigment of the animal pole was slightly moved, and this pole appeared light brown in color. In this case the size of the blastomeres at the animal pole was slightly larger than the normal (Figs. 1, 2). Most of the eggs developed normally. But in some cases, the upper pole was greyish brown even in the blastula as well as the gastrula stages, and the secondary invagination began at the animal pole slightly later than the normal process of gastrulation, which was beginning at the grey crescent in the normal position

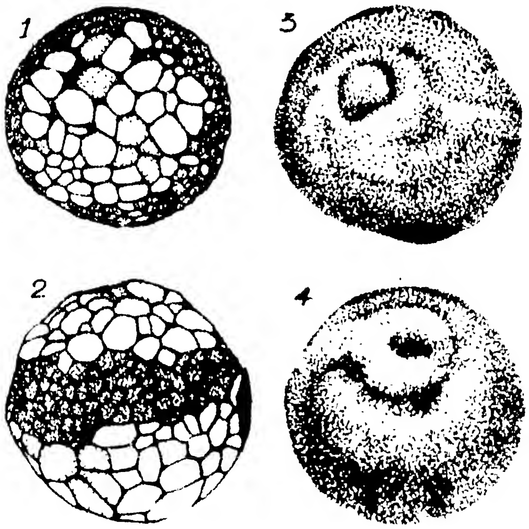


Fig. 1, blastula of the centrifuged egg of the toad, polar view showing the light grey field at the animal pole. Fig. 2, the same egg, lateral view showing the discontinuous change of the size of blastomeres. Figs. 3, 4, gastrula of the centrifuged eggs showing the secondary invagination at the animal pole.

of the same egg. As the result, the invagination occurred at both the animal and the vegetal sides of the same egg (Figs. 3, 4, 6, and 7). The directions of invaginations were opposite to each other.

The relation between the stratification of the egg cytoplasm and the cellular differentiation in the gastrula stage was studied comparatively in serial sections. In the cytoplasm of the centrifuged egg, the centripetal lipoid layer was most distinct (Fig. 5). The layer contains diffusedly a small amount of melanine granules. And, it was clearly partitioned with a thin layer of melanine granules from the underlying yolk layer, which contains the nuclei and occupies

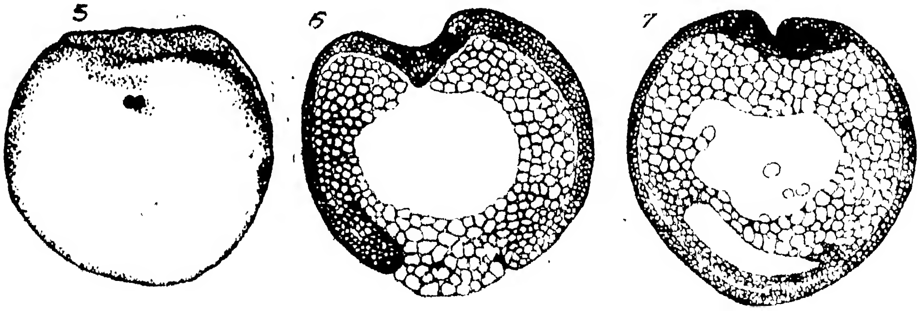


Fig. 5, section of the centrifuged fertilized egg at the stage of karyogamy showing the centripetal lipoid layer and the adjacent layer of pigment and cortical cytoplasm. Figs. 6, 7, gastrulae of centrifuged eggs showing the secondary invagination at the animal pole.

the greater part of the egg. The superficial pigment layer, the cortical cytoplasm covering the surface of the endoplasm, was thin at the centripetal as well as the centrifugal regions, and it was thick at the equatorial region. It was also observed that a portion of the cortical cytoplasm with the pigment granules was shifted to the centripetal side, and as the result, the superficial layer became thick sharply at the border between the lipoid and the yolk layers (Fig. 5). At the blastula stage, single blastocoel was formed. The cytoplasm of the cells of the region of the animal pole, the centripetal pole, contained a large amount of the lipoid and pigment granules, which are derived from the lipoid and pigment layers of the centripetal region. The cells surrounding the lateral as well as the bottom of the blastocoel contained mainly the yolk granules and a little amount of pigment.

At the gastrula stage, depression from the animal pole occurred in the light brown region of the animal pole of the centrifuged egg. In many cases, the depression began at the margin of the lipoid layer in such a manner that the central portion of this layer was protuberant as if it was the "yolk plug". In sections the cells at the region of the secondary invagination was as small as that of the equatorial region, but the former appeared porous because the lipoid had been dissolved away with the solvents. If the eggs were preserved carelessly, the structure of the cells would be destructed easily. The cells of the animal pole invaginate vertically at the beginning, and formed the anlage of the secondary archenteron (Figs. 6, 7) And later, the secondary archenteron grew into one side of the secondary blastopore, and it lay between the ectoderm and the endoderm. It is a noticeable fact that in the course of the secondary invagination at the animal pole the mesoderm as well as the notochord was never formed as far as in the present writer's cases. Accordingly, the origin of the mesoderm of the secondary embryo will be in the mesoderm of the primary embryo.

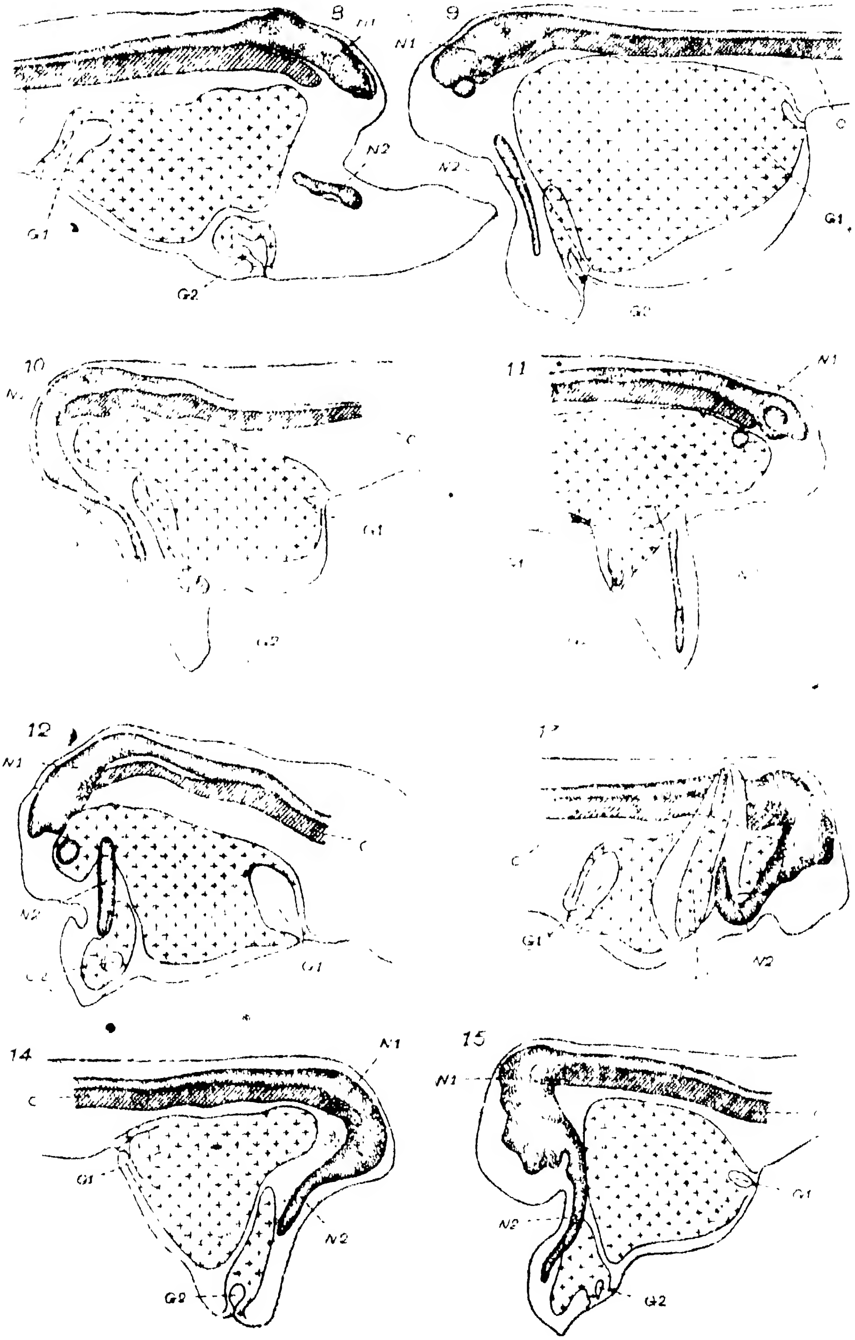
III THE STRUCTURE OF THE DOUBLE-TAILED EMBRYOS

A) The guts.

The guts were formed separately from the primary and the secondary invaginations. Usually they did not fuse each other. The gut of the primary invagination was built up with large cells rich in yolk granules as in the normal embryo. The gut of the secondary invagination, on the other hand, was composed of small prismatic cells, which contain the lipoid and pigment granules. In the majority of the cases the secondary gut was a tube in form with an opening of the gut cavity at the secondary blastopore, and it was composed of the prismatic epithelium of the differentiated intestine. In some cases the cuticle was observed at the inner surface of the cells of the secondary gut. Those facts show that, the secondary gut was derived from the cells of the centripetal region, and that the differentiation of the gut was not affected by the composition of the metaplasmatic granules such as the yolk of any type, or lipoid, pigment and others.

B) Neural tube.

Neural tube was observed in all cases



Figs. 8—15, structure of double tailed monsters reconstructed from serial sections, showing the relations of the guts and neural tubes of the primary and the secondary embryos; G1, G2, guts of the primary and the secondary embryos respectively; N1, N2, neural tubes of the primary and the secondary embryos respectively; C, notochord of the primary embryo.

of the secondary invagination. In some cases the secondary neural tube was independent to the primary, but in other cases, the two tubes fused at the anterior end. And as the result of the latter case, the differentiation of the brain was usually inhibited. It was a remarkable fact that the dorso-ventral axis of the secondary embryo developed at the beginning in coincidence with that of the primary embryo. In other words, the neural plate of the secondary embryo was formed at the dorsal side of the egg, although the direction of the cranio-caudal axis of both embryos was inverse to each other. This shows that the direction of the dorso-ventral axis of the secondary invagination is determined by the original dorso-ventral axis of the egg.

C) Mesoderm.

As mentioned above, the differentiation of the notochord was not accompanied with the secondary invagination, while in the normal as well as in the primary embryo of the double tailed monster it is the case. But as the induction of the neural tube was observed in all cases of the secondary embryo of the double-tailed monsters, the capacity of induction must be due to the secondary invagination, the secondary gut. In some well developed double-tailed embryos, myotomes were observed at both sides of the secondary neural tube. But the cells were easily distinguished from the secondary gut, and they showed similar structure to the mesoderm of the primary invagination. Therefore, it is probable that the origin of the mesoderm of the secondary embryo will be in the mesoderm of the primary embryo.

IV. DISCUSSION

As mentioned above, the secondary invagination was caused by centrifuging the egg of the toad. In this case the cells of the animal pole developed into the endodermal tissue, the prismatic epithelium of the gut, notwithstanding their presumptive meaning. It is also evident that the endoplasmic materials of the egg did not determine the fate of the cellular differentiation, because the cells of the secondary gut have no yolk granule, which is abundant in the cells of the primary gut. Next, it is a well known fact in the amphibian egg that the place where the invagination took place, became the center of organization. And, because in the above mentioned experiments the invaginations occurred in two different regions, the organization center became two in one egg. But it is only a fact common to both organization centers that the ectoderm was underlaid with the invaginated cells, although the development of the invaginated cells is different becoming a gut or notochord. The capacity of induction may be, therefore, caused by the activity of the cells, but not by a special substance. The present writer is inclined to believe that the induction substance, if any, will be produced as a result of the activity of the cytoplasm of the invaginated cells.

The activity of the cytoplasm will be increased, if the cell contains a large amount of protoplasm instead of deutoplasm. And if we assume that the cortical cytoplasm is mainly composed of the protoplasm, then the cytoplasmic activity will be large at the place where the cortical cytoplasm is accumulates in a natural or in an artificial way. According to the writer's observations, the secondary invagination was observed in the centrifuged eggs of the toad in which the cortical cytoplasm was accumulated beneath the centripetal lipoid layer. And as the cells of the secondary gut contained a large amount of lipoid and pigment granules, it is evident that they will have the majority of the cortical cytoplasm which came together to the subpolar region by the effect of the centrifugal force. In the centrifuged eggs of the sea urchins, also, the centripetal pole became the vegetal pole, if the cortical cytoplasm accumulated to the centripetal side (Motomura 1946, 1948). Those facts show that the physiological dominant of the egg cytoplasm in Child's sense (Child 1924) is mainly determined by the relative volume of the cortical cytoplasm in the blastomeres.

It has been advocated by Dalcq and Pasteels (1937) that the morphogenetic potential of the parts of the amphibian egg is determined by the mutual action of the cortical cytoplasm and the yolk. And they drew a schematic figure of the distribution of the morphogenetic potential on the bases of their calculation. Their scheme showed a remarkable coincidence with the map of anlage of the amphibian egg offered by Vogt (1926, 1929). Contrary to them, it has already been maintained by the writer that the cortical cytoplasm is the most important factor for the determination of the embryonic axis (Motomura 1933, 1935). The facts that the position of the dorsal lip has been firmly determined before the determination of the direction of the invagination (Penners and Schleip 1928, Motomura 1935) and that the direction of invagination can be more easily changed than the position of the dorsal lip, will show the importance of the cortical cytoplasm on the determination of the first step of development.

There have been numerous attempts to find a mechanical explanation of gastrulation. Several kinds of models have been constructed that turn in when the arrangement are such that the inner surface of one part of the wall absorbs fluid from the interior faster than the outer wall (Spek 1918, Lewis 1947). It has been shown by the centrifuging experiments that the cortical cytoplasm is viscous in comparison with the endoplasm (Motomura 1933, 1935, 1941). And because the cell boundary between the blastomeres is newly-formed in the course of cleavage, the cortical cytoplasm is not carried into the inner part of the embryo, but remains always on the surface of it, even at the beginning of gastrulation (Motomura 1935). Accordingly, if we assume the difference of velocity of hydration between the cortical cytoplasm and the endoplasm, the mechanism of invagination will be explained with the models constructed by the authorities. And in fact, it was shown by the writer that the invagination took place at the point where the thickening of the cortical cytoplasm was forced by centrifuging in the eggs of the sea urchins and of the toad. It must also be noted that the sudden change of the thickness of the cortical cytoplasm will help the invagination. In the above mentioned cases of the secondary invagination as well as in the normal dorsal lip the size of the blastomere shows a sharp difference in the upper and the lower region of the lip.

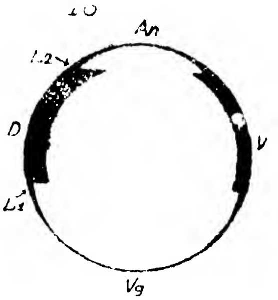


Fig. 16. Scheme showing the relief of the cortical cytoplasm in the centrifuged egg of toad. L1, position of the primary dorsal lip; L2, position of the secondary invagination; An, animal pole; Vg, vegetal pole; D, dorsal side; V, ventral side.

In short, the phenomena of the secondary invagination of the toad as well as the artificial alteration of the embryonic axis of the eggs of the sea urchins by centrifuging will be explained by the local difference of thickness of the cortical cytoplasm. The writer thinks that the cortical cytoplasm of the egg is carved in relief so as to determine the position of the embryo, and that the region where the cortical cytoplasm is thickest becomes the point of invagination, the center of organization. The relative difference of the potencies of differentiation in the dorsal and ventral regions of the egg will be also illustrated with the same hypothesis, the difference of thickness of the cortical cytoplasm (Fig. 16).

Accordingly, it is not necessary to suppose the qualitative difference of the region of the cortical cytoplasm. And, for the explanation of the omnipotent development of the cortical cytoplasm it must be concluded that the cortical cytoplasm of the egg is the harmonic equipotential system.

SUMMARY

A detailed observation on the double-tailed monsters obtained by centrifuging the eggs of a toad, Bufo vulgaris formosus were reported. When the fertilized egg is centrifuged before the first cleavage, invagination takes place in the two distant points of the egg: one the normal dorsal lip, which is in the vegetal hemisphere, and the other the secondary invagination at the animal pole. A double-tailed monster develops from the egg. The position of the primary dorsal lip was not affected, even after the materials in the endoplasm had been redistributed by the centrifugal force. The position of the dorsal lip is predetermined in the cortical cytoplasm before the first cleavage. The secondary invagination was caused by the accumulation of the cortical cytoplasm beneath the centripetal lipoid layer due to the specific gravity. The deutoplasm showed no remarkable effect on the differentiation of the endoderm of the primary as well as the secondary guts. The neural tube and the tail were induced by the newly-formed secondary gut at the animal pole. But the notochord and the mesoderm were not formed with the cells of this region. The polarity of the egg will be determined by the local difference of the thickness of the cortical cytoplasm. The region of the egg where the cortical cytoplasm is thickest becomes the point of invagination. It was deduced that the cortical cytoplasm of the egg is the harmonic equipotential system.

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STUDIES ON THE CONJUGATION OF PARAMECIUM CAUDATUM.
I. MATING TYPES AND GROUPS IN THE RACES OBTAINED IN JAPAN.

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(Received September 30, 1948)

INTRODUCTION

Recent investigations showed that each species of Paramecium caudatum consists of subdivisions of mating types and groups (Sonneborn, 1937, 1938; Jennings, 1938, 1939; Gilman, 1939, 1941; etc.). Japanese clones of Paramecium have never been studied from such a point of view as far as known to the writer. In the present paper is given the result of testing of the mating types of the clones of Paramecium caudatum collected from different localities in Japan. Some experiments on the conditions of conjugation were also carried out.

Before proceeding, the writer wishes to express his hearty thanks to Prof. Dr. Isao Motomura under whose guidance these experiments were carried out.

MATERIAL AND METHOD

Fourty three clones of Paramecium caudatum were isolated from the collections obtained from 17 different natural sources in Japan, i.e. 7 from Sendai, 1 from Morioka, 1 from Yamagata, 6 from Kyôto and Lake Biwa, 1 from Nara and 1 from near Kôbe. The stock cultures of these clones were reared in the writer's laboratory since 1947. For rapid multiplications; paramecia were cultured in the rice-straw infusion medium, adding a small quantity of dried yeast powder. Before using, this medium was sterilised and inoculated with a clone of Bacillus subtilis. Precautions were always taken to insure the predominance of this species of bacterium in the culture. Temperature was maintained at 25°C throughout the experiments.

MATING TYPES AND GROUPS OF PARAMECIUM CAUDATUM IN JAPAN

By the method of test of mating types reported by Sonneborn (1938) and Jennings (1938) combinations of 43 clones were tested. And as the result, 38 clones were classified into 4 non-interbreeding groups, each of which is composed of 2 mating types, and 5 clones remained undetermined (Table 1).

Table 1. Mating types and groups, names of clones belonging to them and their natural sources. Underline: Selfing clone.

Group	Type	Names of clones and their natural source
1	1	Lb5, Lb4 (Lake Biwa); Ks7, Ka1, Ka2, Ka5, Kn1 (Kyôto)
	2	Lb6, Lb8 (Lake Biwa), Ka4, Ks1, Ks2, Ks3 (Kyôto); In2 (Sendai)
2	3	Ks6 (Kyôto)
	4	$K_3$1, $K_3$2, $K_3$3 (Kyôto)
3	5	Kn5, Kn6 (Kyôto); Ys2, Ys3 (Yamagata)
	6	Nd2, Nd3, Nd4, Nd5 (Nara)
4	7	Mo2, Mo3 (Morioka); Ns1, Ku1, Ku2, Bim1 (Sendai); Hy2 (Hyôgo)
	8	Fz1, Fz2, Fz3, Fz4, Is4 (Sendai)
Undetermined		Bi1, Ns3, Is3, Is2 (Sendai); Hy1 (Hyôgo)

Although the materials were collected from different localities in Japan as mentioned above, no special tendency of geographically restricted distribution of the mating types and groups were observed. It seems to be probable that some of the remaining 5 clones may belong to the 5th group, but unfortunately, the partners of those clones have not been found. Selfing clones were also found in them, they are 1 clone in group 1, 1 in group 4 and 3 in undetermined clones.

According to Gilman (1941), 5 groups were found in the American species of Paramecium caudatum. The writer has no opportunity to identify the Japanese groups with those of America, and accordingly, the Japanese groups were tentatively numbered in this paper.

EFFECT OF THE CULTURE MEDIA ON THE RATE OF CONJUGATIONS

Before going onto the above mentioned test of the mating types, some preliminary observations concerning the conditions of conjugation were carried out. In the first place, it was noticed that the rate of conjugants were small in the culture media in comparison with those in the sterile tap water. The same result was obtained when two types of the same group were mixed in the ripe culture media inoculated with bacteria but not with paramecia. However, in the culture media from which crystaloids were eliminated by dialysis, the rate of conjugants were nearly equal to those in the sterile tap water (Table 2). In each case, bacterial zoogloea in the fluid were eliminated by filtration. These results show that some of crystaloids in the culture media have an effect to reduce the rate of conjugants.

Next, experiments on the effect of excretion products of paramecia on the rate of conjugants were carried out. Paramecia were suspended in sterile tap water for 10 hours prior to the maturation, and then they were filtered off. The filtrates of two types of the same group were mixed and used for testing the effect of the fluid on the conjugation. Types of the animals were mixed in the filtrates of each group, but neither the accelerating nor the inhibiting effect was observed.

Table 2. Effect of the culture media on the rate of conjugants

Suspension fluid	Exconjugants / Total animals	Rate of exconjugants	Significance of difference from control
Culture media with paramecia and bacteria	$\frac{202}{335}$	59.8 %	$\chi^2=61.59$
Culture media eliminating crystaloids by dialysis	$\frac{342}{401}$	85.2 %	$\chi^2=0.0179$
Culture media only with bacteria	$\frac{247}{372}$	66.4 %	$\chi^2=30.25$
Sterile tap water (control)	$\frac{322}{376}$	85.7 %	—

In the course of experiments, the writer observed that the rate of conjugation was remarkably influenced by the density of animals, therefore, the density of animals was carefully controlled thereafter. The numerical relation between the density of animals and the rate of conjugants will be discussed in a later paper.

DISCUSSION

It has been shown by Gilman (1941) that in Paramecium caudatum, 5 non-interbreeding groups, each of which is composed of 2 mating types, are distinguished. The present writer found 4 groups in the same species in Japan. It was reported by Jennings that the mating groups of Paramecium bursaria were equally distributed in America (1939), and that the Russian group which composed the 4th group of that species, was quite different from those of America (1944). Although the Japanese groups have not yet been identified with American groups, it is probable that some of the former will coincide with the latter. However, further studies are necessary to find the relation between Japanese and American groups.

SUMMARY

An investigation on the genetic interrelations of 43 clones of Paramecium caudatum collected from 17 different natural sources in Japan was carried

out. In these clones, 4 non-interbreeding groups, each of which is composed of 2 mating types, were distinguished. Five clones were ascertained as belonging to none of these types. Of these 43 clones, 5 were self-conjugating clones, 2 of which belonged to the 1st and the 4th group respectively, 3 were out of the four groups. No special tendency of geographically restricted distribution of the mating types or groups were observed.

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SCIENCE REPORTS OF THE TOHOKU UNIVERSITY, 4TH SER.
(BIOLOGY), VOL. XVIII, NO. 2, 1949.

STUDIES ON THE CONJUGATION OF PARAMECIUM CAUDATUM.
II. INDUCTION OF PSEUDOSELFING PAIRS BY FORMALIN KILLED ANIMALS.

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(Received September 30, 1948)

INTRODUCTION

Recent investigation by Metz (1947) on Paramecium aurelia reported that pseudoselfing pairs were induced by the formalin killed animals of opposite mating type of the same group. This is a new method of analysing the mechanism of conjugation. In the present study, experiments on the mating types of Paramecium caudatum were carried out by the method of Metz.

The writer wishes to express his hearty thanks to Prof. Dr. Isao Motomura for his supervision throughout this work.

MATERIAL AND METHOD

For the experiments, 8 clones of different mating types from the writer's stock cultures, reported in the previous paper, were used; they are, $K^{A}7$ (type 1) and $L^{B}6$ (type 2) of group 1, $K^{A}6$ (type 3) and $K_{B}2$ (type 4) of group 2, Kn6 (type 5) and Nd5 (type 6) of group 3 and Mc3 (type 7) and Is4 (type 8) of group 4. Culture method and other conditions for experiments were the same as those described in the previous paper (Hiwatashi, 1949).

RESULTS

Many individuals of a mating type of Paramecium caudatum were killed by formalin and washed thoroughly with tap water. The killed bodies of the paramecia were mixed with the living individuals of the opposite type. When the reaction was successful, the living individuals sticked to the killed bodies. The writer examined the reaction in all combinations of the above mentioned 8 types, and ascertained that there is a remarkable tendency of the reaction. When the living animals of mating type 2 were mixed with the killed animals of mating type 1, the former clumped with the latter. But on the contrary, living animals of type 1 did not clump with killed animals of type 2. The positive reaction was also obtained between living animals of the type 4, 5 and 8 and killed animals of the type 3, 6 and 7, respectively, but in the reciprocal combinations the results were negative. And at this time, pairs of living animals as well as single animals were released from the clumps. This shows that the killed animal induced the pseudoselfing conjugation. The pairs underwent nuclear reorganizations. It is noteworthy that the pseudoselfing conjugation occurred within two or three days even in the above mentioned reciprocal combinations, in which the mating reaction between living and killed animals were never observed.

On the conditions of clumping and pseudoselfing pairing, some observations were performed. The clump formation of living and killed animals was possible only when the latter was killed by formalin of which concentrations were about 1.5% to 6%, and above or below these concentrations where paramecia either shrunk or formed the hyalin vesicle, it was inadequate for the experiment. However, the clumps were formed even with half-cytolised dead animals, if a large portion of cell surface remained with their normal form. Washing with distilled water after treatment with formalin did not prevent the clump formation but when heated to 100°C in formalin or in the washing water, killed animals failed to induce the mating reaction. Animals killed in a maximal feeding or in inanition, or formalin killed conjugants did not induce either mating clumps or pseudoselfing pairs, but formalin killed mating clumps induced the mating reaction with living animals and the following pseudoselfing pairs. In the mixture of killed animals of opposite mating types, no indications of sticking or clumping were observed, and this suggests that the sticking or clumping is not a simple mechanical phenomena.

DISCUSSION

The above mentioned experiments suggest that the conjugation of Paramecium can be induced by non-living substance, which is produced by the mature individual. And this substance is insoluble in water or in culture media, but it is fixed at the cell surface, because the killed and half-cytolysed animals are able to form clumps with the living animals when the cell surface is preserved in good condition. In these respects, the present writer agrees with Metz (1947) who suggested that conjugation effects might be induced by the interaction of mating type substance at the surface of the animals. It is an interesting fact that in each mating group, clumps of living and killed animals should be formed only in a certain combination between them but their reciprocal is impossible, whereas in both types in the living state, there is no indication of morphological differentiation in them.

SUMMARY

The mating reaction of living animals of Paramecium caudatum with the formalin killed animals of opposite mating type of the same groups was tested and it was ascertained that the reaction was possible in all 4 groups of Japanese

clones, that is the living animals of type 2, 4, 5 and 8 with killed animals of type 1, 3, 6 and 7, respectively. But in the reciprocal combinations, 1 with 2, 3 with 4, 6 with 5 and 7 with 8, the mating reaction was negative. The pseudoselfing conjugation was induced by the killed animals of the opposite type of the same group. In this case, the reciprocal combinations were also successful. Conditions of the induction of mating reaction by killed animals were also described.

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OXIDATION-REDUCTION POTENTIAL OF THE SEA SURFACE AND BOTTOM OF ONAGAWA BAY.

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(With 4 Text-figures)

(Received September 30, 1948)

Gillespie 1920 suggested the importance of the reducing intensity of soils for their fertility. Since then numerous data have been accumulated as to oxidation-reduction potentials (abbreviated O.R.P. below) of biological materials. Kusnetzow 1935, Iwlew '37, Hutchinson '38, Nomura and Kokubo '43, and Zobell '37 determined the O.R.P. of water and bottom materials of lakes or sea, but their measurements were made with materials brought to laboratories. Nomura and Yamada '41 studied the O.R.P. of various bottom materials at laboratory and pointed out the importance of influence of experimental conditions and time effect upon the O.R.P. It became clear that, for eco-

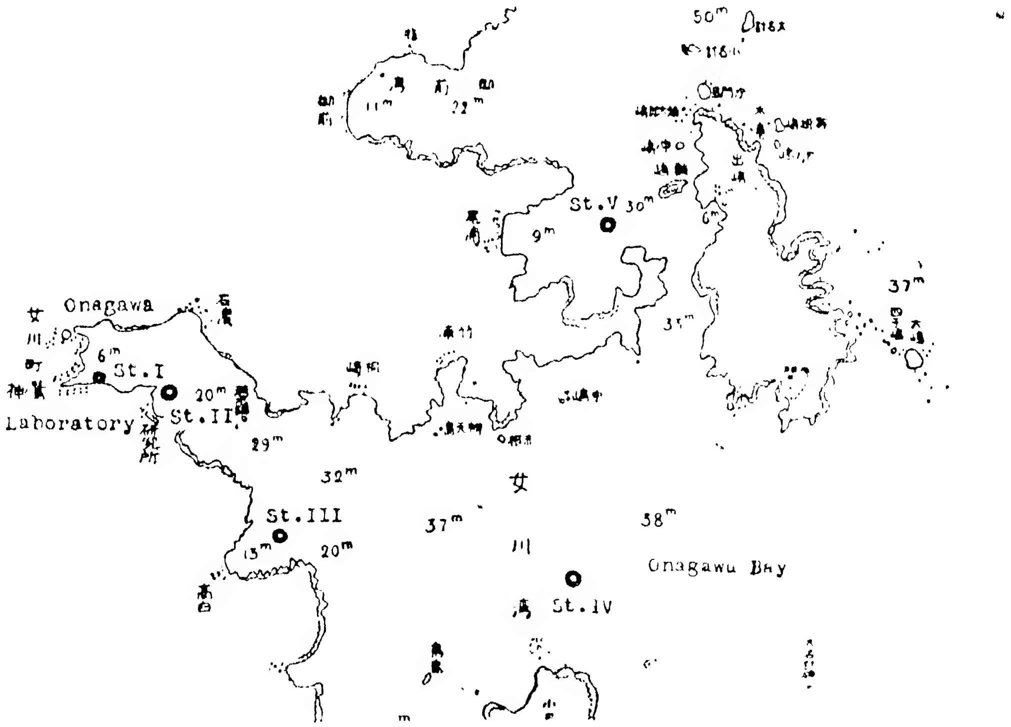

Fig. 1. Chart showing the position of stations of observation.

logical purposes, determination of O.R.P. in situ is highly important. The "disturbed" potential would not be of primary significance. These points were discussed and emphasised in our previous paper ('41). No determination in situ of O.R.P. of sea bottom, however, has been made as far as we are aware (literature appeared during and after wartime abroad being not susceptible to us as yet). The present work was accomplished during the war (1942) aboard the research boat "Koganemaru" of the Oceanochemical Institute of our university at Onagawa, but the result has not been published due to difficulties in publication.

For our special purpose, a special dipping electrode was devised. A platinum platelet was fused on the surface of a piece of glass tubing. The centre of the inner side of the platinum platelet was led with a piece of platinum wire through the glass tube wall to the interior of the glass tube and connected with copper wire. The latter in its turn was connected to a long rubber-coated flexible cord, outside the glass tube. All the connections were carefully protected from contact with sea water and ensuring perfect insulation, with rubber tubing and rubber cement. The glass tube with the platinum electrode was contained in a metal case which has a small window on one side near the lower end, allowing the contact of the electrode surface and the bottom material. The window is large enough to avoid the contact of electrode and the metal case. All the parts mentioned

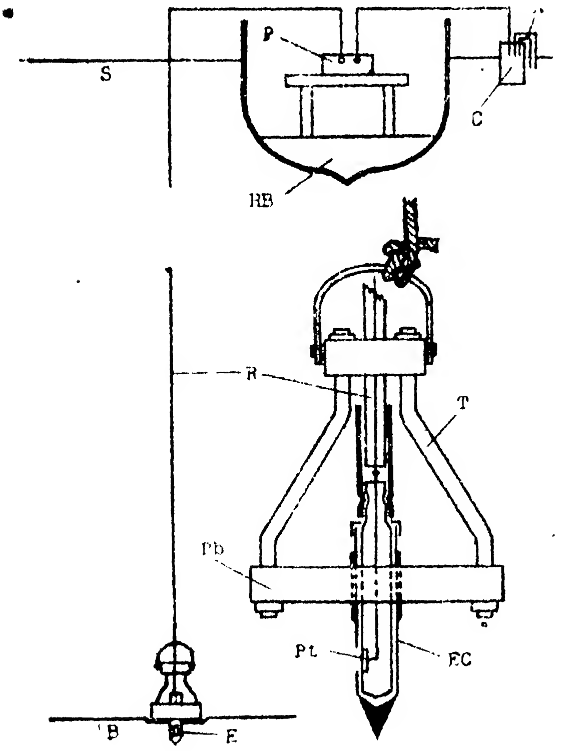

Fig. 2. Equipment of oxidation-reduction potential measurement and construction of dipping electrode.

A.	Agar bridge
C.	Calomel electrode
E.	Electrode
P.	Potentiometer
R.	Rubber coated wire
RB.	Research boat
B.	Sea bottom
S.	Sea surface
EC.	Electrode case
Pt.	Pt-electrode
Pb.	Lead disc
T.	Iron tripod

above are supported by an iron tripod on a heavy lead disc, which ensures the dipping of the electrode into the bottom material. As the leading electrode or reference electrode a saturated calomel electrode was used. It was contained in a wooden box, together with a vessel with saturated KCl solution, and was floated on the surface of the sea. The latter solution was led to the surface sea water by means of an agar bridge. Both the platinum and calomel electrodes were led to the potentiometer on the table aboard the research boat "Kogane Maru", where measurements of O.R.P., pH, temperature, specific gravity, transparency, etc. and sampling of sea water and bottom materials were carried out. Determination of pH was made colorimetrically, oxygen and chlorine were determined at the Onagawa Laboratory, and other analytical work was carried out at the biological laboratory at Sendai. Organic carbon content was estimated by potassium permanganate method after treatment with strong sulfuric acid, and the value obtained was multiplied by the factor 1.8 to give the humus content. Mechanical analysis of bottom samples was made by means of sieves, but grains smaller than 0.5 mm. size were not classified. Loss on combustion was determined with the whole samples and also with parts of them consisting of grains smaller than 0.5 mm. size, as some samples contained fragments of vegetable matter which had not been disintegrated.

Table I. Percentage composition of bottom material in mechanical analysis.

Station	I	II	V	IV	III
Grains > 4.0 mm	23.6 %	23.2 %	2.1 %		0 %
> 2.0 mm	5.8	6.8	1.9		0
> 1.0 mm	4.9	8.6	3.6		0.6
> 0.5 mm	14.7	11.4	3.4		4.1
< 0.5 mm	51.0	50.0	89.0		95.4

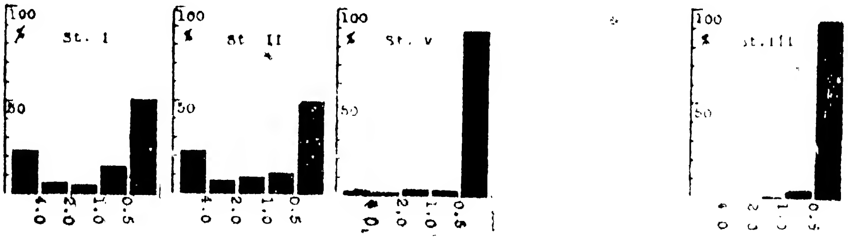

Fig. 3. Diagram showing the composition of bottom material.

Observations were made on October 5 and 6, 1942, at five stations in Onagawa Bay, indicated in fig. 1. Station I is the nearest to the town of Onagawa, which is situated in the innermost of the bay, and at the point where the research boat is usually anchored. The bottom is muddy and decaying matters were found near by. Hydrogen sulfide was perceivable. Station II is in front of the laboratory, and the sea water was considerably clear and the bottom was muddy. Shell fragments were found sporadically. Station III. The sea water was clear, but transparency was estimated at 6.3 M. This was probably because the sun set was nearing and the station was in the shade of hills. The bottom was sandy or rock-sandy in some parts near the station, but at the point of measurement it was sandy. Minute fragments of shell were found. Station IV. Among the stations, this gives the greatest depth: 41 M. The bottom should be sand-muddy according to the chart, but the sampler got out of order and sufficient bottom samples could not be collected, and we could not confirm the bottom character. Station V. The bottom was mud-sandy, fragments of decaying sea weeds and shells were observed.

Table II. Summarized table of data.

Station	I		II		V		IV		III	
	Surface	Bottom	S**	B**	S	B	S	B	S	B
Date and Hr.	6/X	12.40	6/X	11.45	5/X	14.50	6/X	10.25	5/X	17.10
Depth		9.5 m		19 m		22.5 m		41.5 m		22 m
Bottom material		M.		M.		M. S.		S. M.		S.
Forel's Scale	6		5		5		4		4	
Transparency	4		9.5		12.5		20.5		6.3*	
Spec. Grav.	1.235		1.231		1.233		1.235		1.235	
Water temp.	22.4	22.2	22.6	22.3	22.6	21.8	22.5	21.5	22.2	21.8
Temp. of Bottom material		22.3		22.3		21.7				21.9
Cl', ‰		18.87		18.92		18.86		19.08		18.87
O_2, cc/L		4.76	5.06	4.60	5.23	4.61	5.03	3.73	5.23	4.60
pH	8.25	8.27	8.27	8.30	8.25	8.27	8.35	8.27	8.30	8.27
Eh, mV	133	-64	148	-12	128	73	273	108	133	255
rH	20	14	21	15	20	18	27	19	20	26
Loss on Combustion %		17.3		15.3		16.7				14.3
The same in constituents (<0.5 mm)		10.4		16.6		15.4				11.3
Carbon, mg/g		65.6		39.2		50.3				12.4
Humus, mg/g		119		70.0		90.6				22.6

* See the text. ** S=Surface B=Bottom

The results of investigation are given in tables and figures, and brief explanation will be made. In Table II, the stations are arranged in an ascending order of O.R.P. of the bottom.

The depth of the bottom studied ranged from 9.5 to 41.5 meters and shows no apparent relation with O.R.P. The colour of the water varied from 6 to 4 of Forel's scale and shows descending order against the ascending order of O.R.P. Transparency shows also an ascending order, except at St. III which was mentioned above. This might be of significance and would be interesting to be studied more in detail. The deeper the bottom, the lower is the temperature of the bottom material. The content of dissolved oxygen at the bottom showed no obvious relation with O.R.P. of the bottom material and seems to be worth reinvestigation. This fact does not, however, seem to be incompatible with previous knowledges, if we consider our observation that the O.R.P. of the bottom material and that of the supernatant water layer may differ greatly according to circumstances (Nomura and Yamada '41). It is also to be pointed out that O.R.P.s of the surface and that of the bottom are not parallel. The surface O.R.P.s fluctuate in some way unknown and no direct relation with oxygen content was observed.

The O.R.P. values in the table are accompanied by their respective rH values, as regards the meaning of which the reader, if not familiar, is referred to the writings of Michaelis, Nomura or Wurmser.

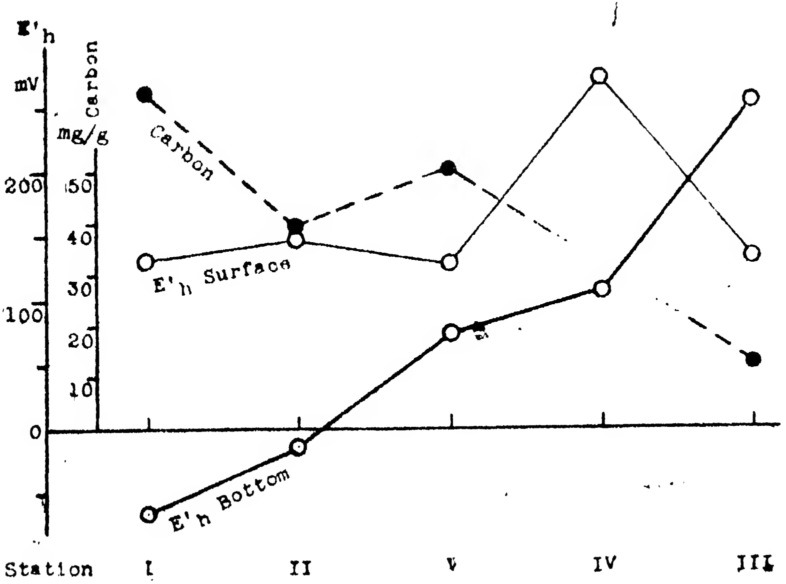

Fig. 4. Graph showing relations of oxidation-reduction potential and organic carbon content of bottom materials.

Largely speaking, the O.R.P. of the bottom varies inversely with the carbon and humus content of the bottom, except a slight discrepancy at St. II. Organic matters on the bottom undergoing decomposition consume oxygen and O.R.P. will decrease. Oxidation of organic matters, however, cannot always directly utilise molecular oxygen, and there are several stages in oxidative processes, in one of which molecular oxygen is activated by some enzyme and can be brought into action. The O.R.P. is a measure of the activity of participating constituents in the O.R. system. The relation of dissolved oxygen, which is molecular, and the O.R.P. is, therefore, indirect and the larger oxygen content is not always accompanied by a higher O.R.P., although it is so in general. One point which is incomprehensible is that the bottom potential was much higher than the surface potential at St. III. Whether this is due to an experimental error or was caused by unknown factor worthy of reinvestigation we can not say at present. The surface potential at St. IV was also exceptionally high, and we can not find any reason for it. Except these few points above mentioned, all the data obtained in the present study seem to be in accord with one another and satisfy our expectation. The only regret is that the bottom fauna of these stations has not been made out, and we could not discuss these data in relation with it.

We should like to repeat and to lay weight upon the importance of the O.R.P. of the bottom, particularly measured in situ, as an ecological factor determining the productivity of sea and inland waters, in accord with Gillespie who suggested the importance of the reducing intensity of the soils for their fertility, quantitative expression of reducing intensity being O.R.P.

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STUDIES ON PHYSIOLOGY AND ECOLOGY OF PLANKTON.

I. HYDROGEN ION CONCENTRATION AND OXIDATION-REDUCTION POTENTIAL IN A CLADOCERAN CULTURE.

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(With 1 Text-figure)

(Received September 30, 1948)

INTRODUCTION

Although various methods have been advanced for culture of daphnids (Banta, 1921; Galtsoff and others, 1937), there have been few studies concerning the physico-chemical conditions of the culture medium. Recently Matsudaira (1943) studied the biochemical factors of clover-extract acting upon the growth of Daphnia and Scenedesmus, the latter being supplied as chief food to the former. His work ranged only for such short period as a week or so in culturing them.

Little has been reported in respect to oxidation-reduction potential (redox-potential) in culture media except the bacterial culture, as to which many contributions have been made. Knight and Fildes (1930) reported that "the spores of B. tetani will not germinate at all, in an otherwise favourable medium, when the redoxpotential is more than Eh 0.11 Volt (pH 7.0-7.65)". Hewitt (1931) showed that the potential difference in various kinds of bacterial culture was due to the difference in peroxide-forming ability. From the results of Gillespie and Rettger (1938) it was observed that there existed obvious potential difference between the two types of Lactobacilli.

In protozoan culture, Efimoff and others (1928) studied the effect of rH upon the multiplication of some species. They employed "platinized" platinum electrode instead of "blank" one, which is usually employed.

.The present report is a part of the series of papers on the physiology and ecology of Plankton. Innumerable works on Plankton have been published, but the fields of studies just mentioned have remained rather unexplored. The aim of this study is to make out the physico-chemical conditions of the culture medium of a daphnid in course of long-period experiment.

The changes in numbers of the animals and of the algae were observed in course of 90 days. In this connection, the changes of pH and Eh in the culture medium were also determined.

I wish here to express my sincere thanks to Prof. Shichiroku Nomura for his kind guidance and criticisms during this study, and also to Dr. Tametake Nagano for his kind suggestions.

MATERIAL AND METHOD

The material used for this study was Simocephalus vetulus (O.F. Müller) which had been cultured in the laboratory for two years. The animals were fed with a green alga, Scenedesmus obliquus Chodat & Artari, which was allowed to multiply abundantly in the culture medium. One per cent extract of mixture of two parts by weight of rice-straws and one part of leaves of pumpkin was prepared, sterilized and preserved as stock extract. In a glass vessel (250 cc. capacity), 10 cc. of the extract were put and added with tap water up to 200 cc. (original culture medium).

At first 10 adult females of daphnid were cultured in this medium with a few number of the algae. This original medium was not changed throughout the course of the study, but 10 cc. of the stock extract were added to it at every 5 days, to compensate the amount used for the measurement of pH. So the amount of medium remained nearly the same. The total number of the living animals was counted, removing them individually in another vessel with a pipette.

The pH value of the medium was determined colorimetrically.

Then the potential measurement was carried out by the compensation method as usual, employing a saturated calomel electrode and a blank platinum electrode, which was immersed in the middle layer of the medium. The potential value thus measured was corrected for the normal hydrogen electrode and was designated as Eh.

At last the estimation of the number of the algae was made by means of a haemacytometer. After stirring the medium to obtain an uniform distribution of the algae, a few drops of the culture collected at random were examined and the numbers per cubic millimeter were counted three times in succession. These values enabled to calculate the total number of the organisms in the whole medium (200 cc.).

After these observations, the animals were returned back to the original medium and then, as previously noted, 10 cc. of the stock extract were added to it. Two such culture vessels were set up for this study and the observations were made at every 5 days from July 27 to October 25 (1946). These culture vessels were placed in a double-glass-walled air bath regulated not to fall below 23°C., while the room temperature varied between 30°C. and 17°C. Correction of the calomel electrode for temperature was made.

In some cases redoxpotential was measured again immediately after stirring the medium: disturbed potential, which will be mentioned later again.

EXPERIMENT AND RESULT

The averages of the observed values are shown as follows (Table I, Fig. I):—

Table I. Averages of the experimental values.

Days	Numbers of Scenedesmus	Numbers of Simocephalus	pH	Eh (+, mV)	rH	Disturbed-pot., Eh (mV)
0	*	10	8.0	401	29.4	-
5	2528×10^6	68	8.2	304	26.5	
10	16312 〃	94	9.2	237	26.3	--
15	4124 〃	117	8.2	291	26.1	+301
20	2112 〃	52	8.3	260	25.3	—
25	8352 〃	122	8.3	256	25.1	
30	9464 〃	82	8.3	255	25.1	+265
35	14124 〃	108	8.2	255	24.9	
40	33388 〃	183	9.0	247	26.2	
45	14400 〃	356	8.1	262	24.9	
50	12248 〃	346	8.1	260	24.9	
55	7586 〃	153	8.3	305	26.8	+293
60	9976 〃	96	8.3	303	26.7	
65	7288 〃	128	8.3	302	26.7	
70	6278 〃	128	8.2	315	26.9	+325
75	1876 〃	76	8.0	330	27.0	
80	1788 〃	44	8.0	330	27.0	+334
85	6684 〃	38	8.5	298	26.9	-
90	6360 〃	20	8.2	312	26.8	-

* The culture medium was inoculated with undetermined numbers of Scenedesmus.

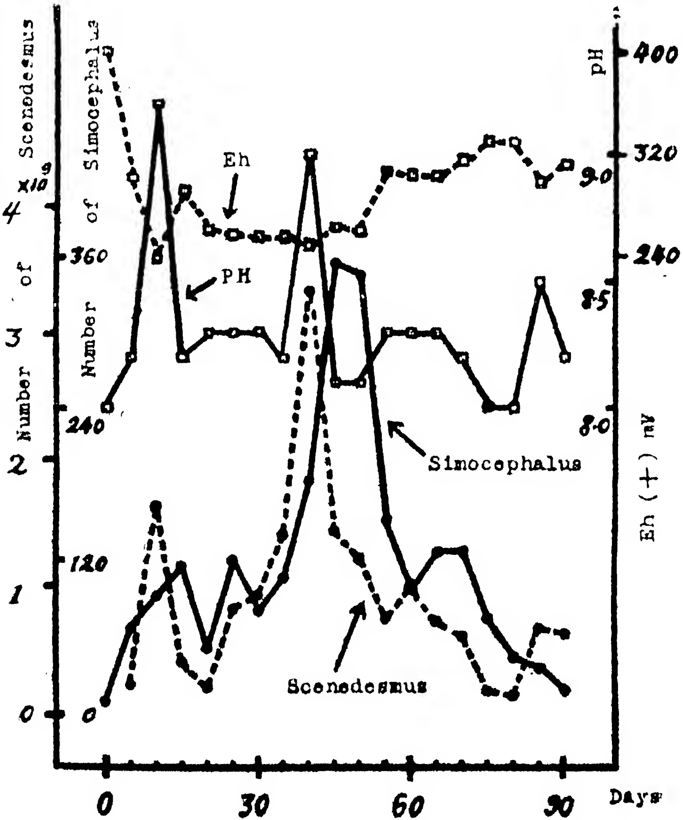

Fig. 1 Changes in populations of Scenedesmus and Simocephalus, pH and Eh of the culture.

DISCUSSION AND CONCLUSION

Matsudaira describes that "the death-rate of Daphnia, feeding upon Scenedesmus in the water, to which clover-extract was not added, increases when its pH-value rises above 9, due to the photosynthesis of Scenedesmus". He attributes the rise of death-rate to the high pH value of the medium. In the present study, it is clearly observed in the figure that there exists an intimate resemblance between the population-curve of the algae and that of the animals (correlation-coefficient r is 0.81, highly significant at the 0.1% level), with a time lag. This indicates that the number of the latter depends upon the food content of the culture. Scenedesmus was found in the alimentary canal of the daphnid dissected under the microscope. Noland (1925) said that the nature and amount of the available food were the essential factor for the distribution of the fresh water ciliates, both in the field and in the laboratory. The pH value of the medium was 9.2 and 9.0 on the 10th and the 40th days of the present experiment, but the number of the daphnid was found much increased on the next following observations, and we can not consider the high pH value of the medium as responsible for high death-rate of the animals as Matsudaira maintained in his experiment. The number of Scenedesmus was great on the days above mentioned and the daphnid was found enormously multiplied on the next observation. We therefore rather agree with Noland in laying weight upon the amount of food as a factor determining the population of the organisms.

While the algae multiplied in the medium, the CO_2 might be exhausted for their photosynthetic action. This is the reason for the high pH at the abundant multiplication of the algae (between the number of the algae and the pH values correlation-coefficient r is 0.67, highly significant at the 1% level).

Judging from the fact that the disturbed potential measured immediately after stirring the medium is only about 4 mV, on an average, higher than before, one is led to conclude that quantities of O_2 absorbed from the air should be very small. It is acceptable, therefore, that the effect of O_2 upon the potential is slight in this system. The changes of pH are caused mainly by the CO_2-content of the medium, which is affected by photosynthesis.

On the other hand, the potential did not always show any constant value at the same pH, especially in the later course. In the course of long-period culture the influence upon the potential of the waste products, which accumulate gradually in the medium with lapse of time, might be expected. As to the waste products, Grosvenor and Smith (1913) first pointed out that they might be of some unstable character and consequently quickly oxidized or destroyed. Later Stuart and others (1932) supposed that the accumulation of waste products resulted in CO_2-production with subsequent reduction of O_2-tension in the medium.

Using a high concentration of hay infusion (20%) as the culture medium, Efimoff and others found that the medium showed at first very reducing character and after 3-month culture it reached high oxidising intensity, the rH being 35 which remained for more than 5 months. In my experiment the concentration of the medium was 0.05% and the rH values remained in narrow range, namely from 29.4 to 24.9, though these values were chiefly influenced by changes in the numbers of Scenedesmus in the medium as mentioned above. In bacteria-free culture of Chilomonas, Jahn (1933, 1935) observed that the Eh of the medium dropped as the growth of the organism proceeded. In the present result also similar relation would be conceivable between the multiplication of the animals and the decrease of the potential

The decreasing tendency of the multiplication of the algae in the later course was probably due to the weakness of sunlight in the daytime, in spite of ample supply of nutrient by the addition of the stock extract to the medium.

Besides the conditions mentioned above, the influence of numerous microbes, especially that of bacterial flora, should be considered qualitatively and quantitatively in order to clarify the properties of this system as a whole.

SUMMARY

1. The changes in numbers of Simocephalus vetulus and of Scenedesmus obliquus in the culture were observed in course of 90 days. In connection therewith, both the pH and the Eh of the medium were also determined.

2. There was intimate resemblance, with some time lag, between the curves of the animals and that of the algae, showing that the latter were the chief food of the former.

3. Approximate reverse changes were observed in pH and Eh. But in the later course this relation was irregular, suggesting the existence of some substance which influenced the relation in this system.

4. There was also close similarity between the curves of pH and of the algae, which showed that the changes in pH were caused mainly by CO_2-content affected by photosynthesis and respiration in the medium.

5. The slight potential drift measured immediately after stirring the medium was due to aeration of the system. This slight effect of aeration upon potential suggests that molecular oxygen enters into oxidation slowly and indirectly after activation and the oxygen demand of the system is not large.

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SCIENCE REPORTS OF THE TOHOKU UNIVERSITY, 4TH SER.
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STUDIES ON PHYSIOLOGY AND ECOLOGY OF PLANKTON. II. OXYGEN CONSUMPTION AND HEART-BEAT RATE IN DEVELOPMENTAL STAGES OF SIMOCEPHALUS VETULUS (O. F. MUELLER).

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(With 1 Text-figure)

(Received September 30, 1948)

Obreshkove (1930) showed that the respiratory rate in later instars was lower than in earlier instars of Simocephalus exspinosus.

Computing from Obreshkove's data, Terao (1931) proposed an exponential formula for the relation between age in adult form and respiratory rate. Obreshkove measured oxygen consumption at distant intervals and did not study the intermediate stages, and failed to see the fluctuation of respiratory rate in the course of development.

In the present study, the respiratory and heart-beat rates were measured day by day following the developmental stages.

Measurements were made with Nomura's modification of Thunberg's respirometer (Fenn, 1927; Obreshkove, 1930). The capacity of the respiratory chamber was 6 cubic centimeters and the volume of the capillary was 3.85 cubic millimeters per centimeter of the scale. Ten individuals (female) of the same brood were used for experiment up to the 4th instar, and five of them were used for later instars. After each measurement they were transferred back into 200 cc.—culture medium which was described in the previous paper. The green algae, Scenedesmus, which adhered to the daphnid were washed away with distilled water. The experimental temperature was regulated at 25°C + or — 0.01 in respiratory experiment (Exp. A). In counting the heart-beat rate, a glass vessel was used. It was double walled on sides and bottom, and the top was open. Cover glass with a hanging drop containing the animal to be studied was placed individually on a support in the vessel and the latter was covered with a large slide glass. The whole was set on the microscope stage and observation was made with a low power magnification. In the vessel the water was circulated from the thermostat (25°C + or — 0.01)—(Exp. B). Exp. A was carried out in August and Exp. B was in June, 1947.

In Exp. A, the ovary began to produce eggs in the 4th instar, became full of eggs in the 5th instar and discharged them into the brood chamber in which the eggs were developed fully (maturity of mother). Hatching and releasing of

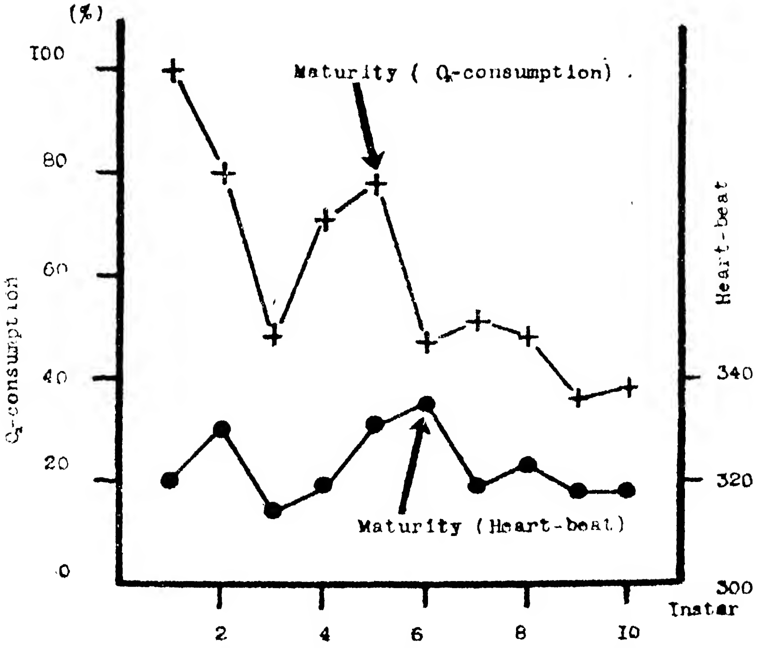

Fig. 1. Relation between metabolism and development

the larvae took place at the end of this instar. In Exp. B, maturity was reached in the 6th instar. The results are shown in Fig. 1. The relative respiratory rate was expressed as oxygen consumed per unit body volume, which was assumed as cube of the body length, and the rate in the 1st instar was taken as 100 per cent.

It is clearly found from the figures that the metabolic rates rise once at maturity and decrease gradually thereafter.

Obreshkove could not observe this rise at maturity, as he did not follow the development step by step. Furthermore it is supposed from the experiments that eggs have a high metabolic activity on one hand, and the rate of mother alone decreases with advance of age on the other.

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THE DISTRIBUTION OF pH IN THE ALIMENTARY TRACT OF EARTHWORMS.

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(With 2 Text-figures)

(Received December 1, 1948)

The pH values of the soil of the habitat of earthworms have been studied by many workers and also in our laboratory. On the other hand, the pH value of the content of the alimentary canal of earthworms has not been determined, and it is particularly desirable to be measured in situ. Thus I tried to determine the pH of the intestinal content of earthworms. Measurements were made potentiometrically with specially devised quinhydron electrodes. This report contains the data obtained in the previous work (1940, unpublished) and those obtained in recent studies (1948). In the earlier work, the syringe electrode (fig. I, ii) was used for Pheretima divergens, and in the later work, the dipping electrode was directly inserted into

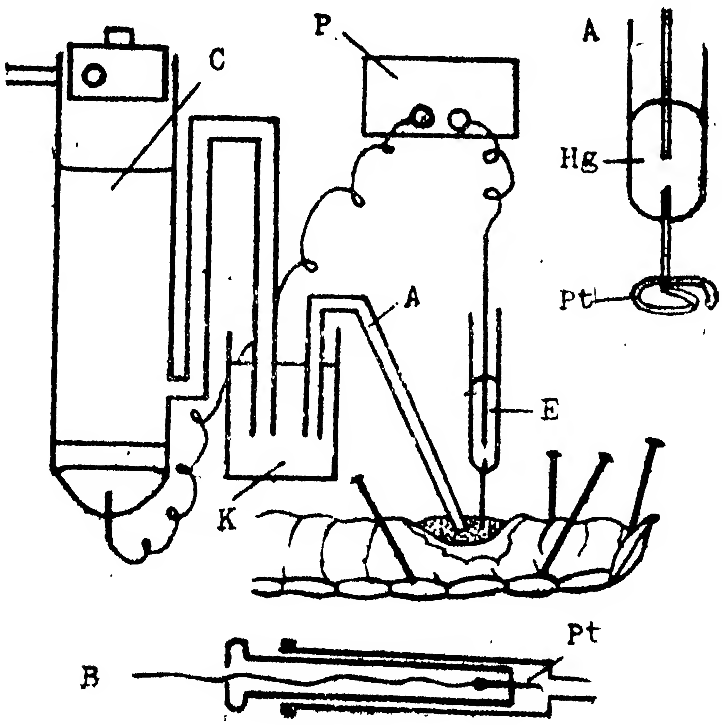

Fig. 1. Diagram of instruments arranged on a dissected earthworm.

P. Potentiometer
E. Electrode
C. Calomel electrode
A. Agar bridge
K. Sat. sol. of KCl

the alimentary canal of Pheretima communissima. The worm was dissected along the body axis after being anesthetised, and the electrode was inserted into the hole made in the digestive tract. In the case of the syringe method, the samples should have been previously removed from their original situation, and their considerable amounts were needed for each measurement. With the dipping electrode, on the contrary, the measurement could be made directly, quickly and in situ where the intestinal content was originally situated.

The results are given in table I. Two maxima of pH value seem to exist in divisions III and VI. The value is low in IV, and also gradually decrease in the posterior parts, VII, VIII and IX. The variation of pH value is rather wide in range and general statement is difficult, and more detailed studies are desirable to be made, in connection with physiological condition of the earthworms.

Table I. Table of the summarized results.

			ex. m.	I	II	III	IV	V	VI	VII	VIII	IX
A	Ph. comm.	Av.	7.31	7.10	7.29	7.41	7.02	7.21	7.47	7.25	7.06	6.99
		(Max.)	7.65	7.13	7.48	7.74	7.21	7.48	(7.48)	7.49	7.46	7.46
		(Min.)	6.86	7.08	7.01	7.01	6.60	7.08	(7.45)	6.96	6.67	6.73
B	Ph. comm.	Av.	7.26		7.63		6.95	7.16		7.27		7.43
B	Ph. div.	Av.	6.18		7.43		7.18	7.05		6.84		6.61

A: Direct method B: Syringe method ex. m.: external soil

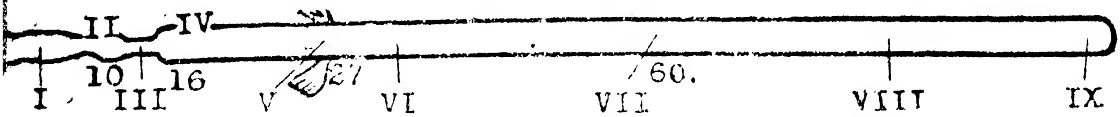

Fig. 2. Diagram of the intestine showing its situations measured. Arabic numbers correspond to those of segments.

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PHYSIOLOGICAL STUDIES ON THE PIGMENTARY SYSTEM OF CRUSTACEA.

III. THE COLOR CHANGE OF AN ISOPOD LIGIA EXOTICA (ROUX).[1]

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(With 3 Text-figures)

(Received September 30, 1948)

INTRODUCTION

The mechanism of chromatic change in the isopod crustaceans has been studied by some authors. Recently Kleinholz (1937) has found a pigment-activating substance in the marine isopod Ligia baudiniana. Ligia shows a diurnal rhythm, being dark by day and pale by night even in constant artificial darkness. Injections of aqueous extracts of the heads of light animals into dark ones brought about lightening by a concentration of the melanophores, as in the shrimps. Hence Kleinholz concludes that the diurnal pigmentary activity is not due to a cycle of exhaustion and elaboration of secretory material in the endocrine gland controlling the color change.

Smith (1938) examined the color change of the related species Ligia oceanica, using a method formerly applied by Hogben and Slome to the investigation of the chromatophores in vertebrates, although he made no injection experiment.

The present work is mainly concerned with the chromatophoric behavior of Ligia exotica (Roux). This investigation was undertaken at the suggestion of Prof. Dr. S. Nomura, whom the author is indebted for his guidance. Acknowledgement is also due to the Oceano-Chemical Institute at Onagawa, Japan, for giving the facilities for collection of the animals.

MATERIAL AND METHODS

The materials for this work were collected by the seashore of Onagawa. The animals were carried to the laboratory in Sendai by train, taking three hours, then turned into a large container in which they could live for some weeks in good condition.

A glass dish about 12 cm. in diameter was used for the observation of the responses of animals to backgrounds of

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[1] This report is a part of the series of papers submitted to the Faculty of Science, Tôhoku University, in partial fulfilment of requirements of the degree of Doctor of Science, March 1948.

various colors. The bottom of this glass dish was covered with a sheet of filter paper, saturated with sea water, and it served as a white background. To make the black background, the filter paper in the glass dish was covered by a sheet of black paper. For the various colored backgrounds, the colored papers were employed respectively in the same manner as the black one. In each dish were placed three individuals together. The containers were illuminated, if necessary, by a 60-watt electric lamp at a distance of 50 centimetres.

It was also necessary, during the course of the investigation, to examine the melanophore behavior of blinded animals. For the blinding of this isopod, extirpation of the sessile eyes was unsatisfactory, because the ensuing hemorrhage caused sometimes death of operated individuals. Then, such operation was eventually abandoned except for the control test, and the blinding was accomplished by covering the eyes with black paint, made of mixture of Canada balsam and Indian ink. This simple method was convenient especially for the selective partial illumination of ocelli of Ligia.

For the recording of degrees of contraction and expansion of pigment in the melanophores, the melanophore index (micron) introduced by Hogben and his collaborators was used. On this scale, pin-point contraction is denoted by 1.0 and maximal expansion by 5.0. Reading of index was taken at the definite point of the posterior region of the body.

For the observation of the melanophore, the animal was supported in a simple apparatus. In this apparatus, the animal was held quiet for long time during the experiment, and the behavior of each melanophore could be observed under the microscope without disturbance.

For the histological study of the eyes of this animal, the head parts were fixed with Carnoy's solution. Then the paraffin sections were mounted on the slide glass, and were stained slightly with Delafield's haematoxylin and 1% eosin solution.

OBSERVATION AND EXPERIMENTS

1. Color Change of the Animal in Response to the Backgrounds.

The chromatic behavior of this animal in the laboratory indicates that the differences of coloration ranging from black to a light mottled grey are mainly due to the amount of light reflected from the background. This will be shown in the experiment about the selective painting of the eyes.

The observations on the responses of the melanophore to the various environmental conditions are summarized in Table I.

On the black background, illumination of the intact animal caused expansion of the melanophores. On the white background, the melanophores were contracted. But it is true only when the eyes are exposed. Animals blinded by painting the eyes with black tint in such a manner as described showed lower degrees of melanophore expansion than the seeing animals on a black background, and considerably higher degrees of expansion on a white background. In darkness, the melanophores assumed intermediate condition and they were

Table I.

Background	Condition of animal	Chromatophore index in different illumination		
		Bright light	Dim light	Darkness
Black	Normal	5.0±0.0	4.6±0.5	2.8±0.2
	Blinded	4.2±0.5	3.8±0.5	2.8±0.2
White	Normal	1.2～2.2	1.0～2.2	2.8±0.2
	Blinded	4.2±0.5	3.8+0.5	2.8±0.2

less expanded than in the case of blinded animal in lightness, the value of the melanophore index being about 2.8. We could not keep the animals at constant temperature throughout these observations, the range of temperature being 18 + or − 2°C. The data given in the Table I are based on a mean value for fifteen animals in each case.

Blinded animals in dim light have a lower melanophore index than blinded animals in bright light, either on white or on black background. Unblinded animals have a lower melanophore index, when illumination is reduced, on the black background, but the reverse is true on the white background.

Next, the reaction of melanophores to the colored background, was confirmed as shown in Table II.

Table II. Chromatophore index of the melanophore on colored backgrounds.

Color of background	Normal animal	Blinded animal
Yellow	1.0±0.2	5.0
Light green	2.0±0.5	5.0
Green	2.5±0.5	5.0
Orange	3.2±0.5	5.0
Red	3.5±0.5	5.0
Purple	4.0±0.5	5.0

The data given in Table II are also based on mean values for fifteen specimens.

2. Time Relations of Chromatic Behavior of Melanophore

When the background to which the animals are adapted is changed, they gradually adapt themselves to the new background. The rates of color change of animals adapting themselves to backgrounds in the diffused light are shown in Fig 1. When from a white to a black background, the time relation curve of color change shows a gradual rise, and when from a black to a white background, the curve indicates a gradual descent, with about the same slope.

The time relations have been determined under further experimental conditions as follows:

a) Color change in animals with

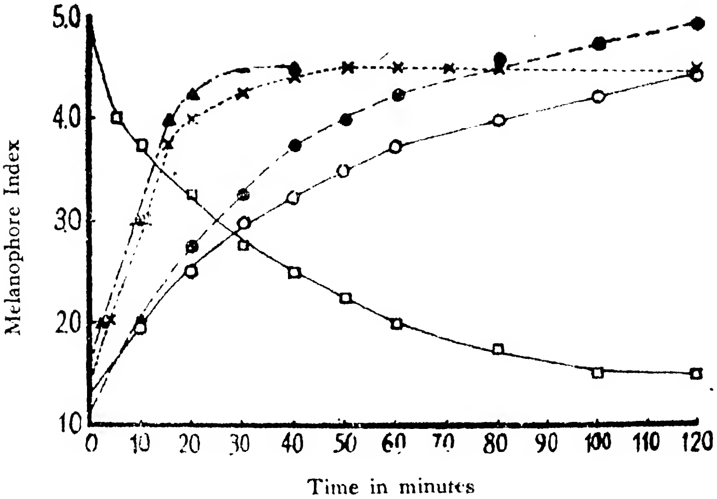

Fig. 1. Time relation curve of color change.

--○-- from white to black background.

--□-- from black to white background.

--● black-painting of the eyes.

...×... extirpation of the eyes.

---▲- injection of CO_2 saturated water in body-spaces.

eyes extirpated on the white background.

A number of Ligia were blinded by removal of the eyes with a spear-point needle. Such specimens, with contracted melanophores, became darker after the operation. The behavior of their melanophores was plotted in Fig. 1. It will be shown that the rate of the expansion of the melanophores in this case is very rapid, compared with that of reaction of normal animals.

b) Color change in animals blinded by painting eyes on the white background.

In this case, the rate of the darkening of the body is quicker than in the normal white adapted animal transferred to the black background.

c) Color change following the injection of CO_2-saturated water in the white adapted animals.

When 0.1 cc. of water, saturated with CO_2, was injected into the light adapted animals, the melanophores expanded more rapidly than in the case of the extirpation of the eyes, and the animals died in about 40 minutes.

3. Partial Blinding of the Eyes

The eyes of Ligia exotica could be divided into: (a) dorsal part (D, Fig. 2) directly exposed to overhead illumination; and (b) a lateroventral part (L and V, Fig. 2) exposed to light reflected from immediate surroundings. By blinding of the dorsal part (D) of the eye only, the animals show the same response as the totally blinded animals on a black background and a lower melanophore index on a white background than either blinded animals on a white background or seeing animals on a black background. With the latero-ventral part (L + V) of the eye blinded, the response on both black and white backgrounds has been the same as in seeing animals on a black background. Table III shows the results of these experiments. The melanophores of the two experimental classes are compared with those of seeing animals (0), and of totally blinded animals (D + L + V).

Table III.

Blinded parts of the eyes	Background	
	Black	White
0	5.0	1.6
D	4.0	1.2
L + V	4.5	4.5
D + L + V	5.0	5.0

When the illumination was limited on the different parts of the eyes, the melanophore index of the animals produced the results as the following figure shows. In this figure the white area is the part stimulated by direct illumination.

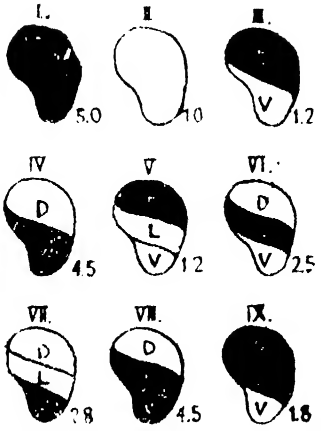

Fig. 2. Chart showing the value of melanophore index when illumination was restricted to different parts of the eye, D, L and V. The parts illuminated are white.

4. Effects of Injection of Aqueous Extracts of the Head

The head parts of the white adapted specimens were crushed in mortar and extracted by filtered normal sea water. When 0.2 cc. of the extract thus obtained was injected into the dark adapted animals, the melanophores were concentrated only to the degree of 4.0 of the melanophore index.

Next, I have treated the extract from animals of white phase at night, whose melanophores were contracted very distinctly on account of the diurnal periodicity. This extract was injected to the dark adapted animal in the same way. It has induced considerable contraction of the melanophore.

5. Comparative Structure of the Eyes and Pigment Migration in the Eyes

The eyes of this animal are considerably complex. The structure of the eyes is shown in Fig. 3 diagrammatically. This type of the compound eyes must be included in the superposition eye which is usually found in the nocturnal insects and decapods.

There are as many as three kinds of pigment cells disposed around a transparent central axis in an ommatidium of this animal. The distal cells contain black pigment which absorbs the light, and pale or colored pigment which reflects the light. The cells themselves do not change in shape or position; the granules of pigment merely become

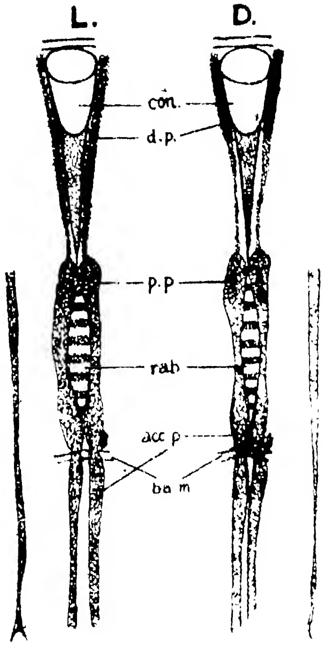

Fig. 3. Ommatidia from the eyes of Ligia exotica, showing the general structure and the position of the three pigments in light and dark phases.

L, from an eye in the light condition;
D, from a dark-adapted eye:

con.	cone
d. p.	distal pigment
p. p.	proximal (retinal) pigment
acc p.	accessory (reflecting) pigment
rab.	rhabdome
ba. m.	basement membrane

clumped or dispersed. Thus in dim light, the pigment in the distal cells is withdrawn upwards and the eye can function as a superposition eye.

During the day time, the pigment in the distal pigment cells is expanded; and since this pigment varies in quantity in different parts of the eyes, these appear striped or spotted. At night, the pigment disperses in all parts and the banding disappears.

The rhabdome as a matter of fact is made by the fusion of the inner differentiated faces of the functional retinular cells whose outer cytoplasmic portions are filled with pigment granules. The pigment of the distal and of the proximal cells consists of melanin particles; hence the pigmented parts of these cells are black in appearance.

The third kind of pigment, the reflecting pigment, is white, at least when viewed by reflected light, and is contained in cells that fill the somewhat irregular spaces between the proximal ends of neighboring ommatidia. It is believed to be made up of guanin particles and is collectively designated as the tapetum. The retinal nerve fibers are inward prolongations from the proximal pigment cells. They pass proximally from the bodies of the cells through perforations in the subjacent optic ganglions. The most remarkable character of the structure in the Ligia's eye is the prolongation of the retinal cells from the basement membrane to lamina ganglionaris.

6. Melanophores in the Larval Stage

In the breeding season, we can see easily many larvae of this animals. But the differentiation of the melanophores was not advanced in such larval animals. I have observed an interesting fact during this investigation; that is to say, when the larvae released from brood pouch were immersed in the normal sea water indefinitely, the pigmentary system on the dermal surface did not differentiate in the melanophores and remained in the state of the ramified networks for the period of laboratory life of the animal in sea water.

On the contrary, the larvae landed from the sea water on the sandy ground, from the first time, showed considerable differentiation of the melanophore, and responsed by dispersion and concentration of the melanophore to light conditions from the early time.

DISCUSSION AND CONCLUSION

In the marine isopod Ligia baudiniana, Kleinholz (1937) has found a pigment-activating substance which has not been studied with regard to the exact localization. Injections of aqueous extracts of the heads into the body space of dark animal brought about lightening as in the case of shrimps. Kleinholz concluded that the diurnal pigmentary activity is not due to a cycle of exhaustion and elaboration of secretory material in endocrine gland controlling the color change.

Stahl (1938) has examined the terrestrial isopods. The extracts taken from light adapted Oniscus, Porcellio and Mesidothea gave a surprising result; injected into blinded Leander adspersus with maximally expanded chromatophores no reaction was observed. But the injection of the extracts taken from dark adapted animals into white adapted Leander caused an obvious expansion of the contracted chromatophores.

Smith (1938) examined the color change of Ligia oceanica, by using a method formerly applied to the work of the chromatophores in vertebrates (cf. Hogben and Slome, '36), but without making any injection experiments. He studied also the time relations of chromatic function; namely, the necessary time for the chromatophores to contract or expand when the background is reversed from white to black and vice versa, and the equilibrium intervals for transition from illumination on a white and black background to darkness and vice versa. According to Smith these time relations show that the background response is controlled by two hormones, one concentrating and the other expanding.

Smith observed also the relation of the different parts of the eyes to the chromatophoral activity, and distinguished two groups in the compound eye. One dorsal group is accessible to direct overhead illumination and is responsible for initiating the discharge of the hormone which brings about melanophore expansion. The other latero-ventral group react to light reflected from the immediate surroundings and is responsible for initiating the discharge of the hormone which evokes melanophore contraction. These observations are of interest, when compared with my own investigations of the relation between the melanophore reaction and the function of the different parts of the eyes in Ligia exotica.

The same will be concluded also from the results of my studies on the pigment migration in the eyes.

The response of the dermal melanophores to illumination is partly direct and partly controlled by the eyes, and it should not be overlooked that there exists coordination between the pigmentary hormone derived from the head and the nervous control through the eyes stimulated by light. But it may be considered from the result of my experiment that the darkening of this animal is sometimes dependent upon the unfavorable condition for activity, because the expansion of the melanophores was induced by the extirpation of the eyes and the injection of CO_2-saturated water into the body.

The structure of the eyes of Ligia must also be taken into consideration for the behavior of the dermal melanophores. From the histological studies on the structure of the eyes and from the development of the melanophore in relation to the environmental conditions, Ligia exotica may be considered as the transitional form from marine to terrestrial form. We must bear in mind that the activity of the melanophores and the phenomena of color change correspond consequently to outer and inner conditions of the animal.

SUMMARY

1. The color change in Ligia exotica (Roux) was studied from the physiological standpoint, and the eye structure and pigment migration in the eyes were also investigated from histological standpoint.

2. The dermal melanophore responds by contraction and expansion to the white and black backgrounds in light respectively.

3. The black painting of the eyes with my own method makes the melanophores expand considerably as well as the extirpation of the eyes.

4. In the darkness or in night, the melanophores contract to the degree of 2.8 + or − 0.2 of melanophore index.

5. The chromatic state of normal and operated animals on the colored backgrounds was studied.

6. The rates of color change resulting from transferring Ligia from a white to a black background in the diffused light and vice versa are shown in the time-relation curve, comparing with the case of the black-painting and the extirpation of the eyes.

7. Injection of CO_2-saturated water into the white adapted animals expanded the melanophores more rapidly than extirpation of the eyes.

8. By partial painting of the eyes, it was possible to test the effect of the selective elimination of the different groups of ommatidia.

9. Effects of injection of aqueous extracts of the head of animal which had been adapted to the white background, into the dark phase animal were not distinct. But the extract of the night phase animal effected on the dark phase animal by the considerable concentration of melanophore.

10. By the histological investigation of the eyes, it will be inferred that Ligia exotica has superposition eyes. And the existence of the pigment migration in the eye indicates that this isopod has the complex structure as higher decapods.

11. The differentiation of the melanophore in the larval stage advanced only in terrestrial life.

12. According to the structure of the eyes and the chromatic behavior of the animals, we can recognize that this isopod may be the transitional form from marine to terrestrial form.

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STUDIES ON THE SCALLOP OF MUTSU BAY.

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Nishioka 1943 and Nishioka and Yamamoto 1943 have already published papers on the ecological and fisheries studies on the scallop of Mutsu Bay. Among the results of their studies, the following facts were observed and pointed out as worthy of notice, from a practical viewpoint of scallop breeding, that the development of the scallop spats varies enormously from year to year according to the sea conditions of the year, and the scallop grows at present only on the gravel bottom, which occupies only a small part of the bottom of the bay along the coast, and the largest part of the bottom consisting of mud is not inhabited by the scallop at all, although in the past the latter area was much more densely populated than the former.

The key of the problem of scallop culture in Mutsu Bay seems therefore, to lie in that whether the vast area of muddy bottom, where scallops grew abundantly in the past, could be utilised as the culture ground for scallop spats, reared in the bay or transplanted from elsewhere. Judging from the facts in the past, it seems not impossible, we believe, that the muddy ground which makes the largest part of the bottom of the bay, could be utilised as culture grounds and would produce as abundant amount of scallops as in the past.

Bearing the view above mentioned in mind, we have been, since 1943, carrying on studies on artificial fertilisation, spat collecting, feeding the young with cultured plankton and other work in relation to practical problems.

The expense of the part of the studies reported here was defrayed by a grant from the Saito Gratitute Foundation, to which we take this opportunity to express our cordial thanks. The publication of this report has been delayed owing to difficulties in publication during and after the wartime. A more detailed report in Japanese will appear in the near future. The studies have been continued and in progress, and since 1947 Aomori Prefecture has taken up the problem of scallop culture and charged the authors with the investigation granting an expenditure. Part of materials used in the experiment was supplied by the Fisheries Experiment Station of Hokkaido, to which we wish to express our hearty thanks.

Spat Collection.

For the purpose of scallop breeding,

sufficient spats should be collected. The sea, especially in the winter, is rough in this district and the collector for oyster or scallop spats used in other localities are not suitable in Mutsu Bay, and attempts were made to find a suitable form of collector. Two types of collectors were, therefore, devised and their effects were compared. Type I. A series of large scallop shells, drilled in the centre, or other materials suitable for settling of spats were connected at intervals by means of a rope, several feet long. Many of such series were suspended from a float, which was kept about 3 feet below the sea surface, being connected with buoys above and with weights below by means of appropriate lengths of rope. Type II. Two rope, about 5 cm. in diameter, were spun horizontally about 5 meters apart and parallel, one above the other in the sea. The upper rope was equipped with buoys at intervals of 10 meters and with weights on the bottom at intervals of 20 meters, by means of similar rope. Both the ropes were also connected by nets or other suitable waste materials to give attachment for spats.

On May 3, 1943, a collector of type I was installed at a point of 13 meter depth and 2 Km. off Noheji, and two collectors of type II were installed at a point of 15 meter depth about 1 Km. off Yokohama. One of them was set at a right angle, and the other set parallel to the direction of dominant waves. On May 13, 1943, a collector of each type was installed at a point of 11 M. depth and 2 Km. off Kawauchi. Buoys of the two latter were stolen and the collectors sank to bottom and the result of the experiment could not be determined. The collectors off Noheji and Yokohama were found satisfactory at the end of August, when the observation was finished. The construction was quite resistant against waves and currents and seemed to fit the purpose. The type II was inconvenient for examination of settling of spats during the whole season, although it might be rather useful for practical purposes. It was thus abandoned in later experiments. While the collectors could stand all the mechanical disturbances in the sea during the season, settling of spats was quite poor. This was not due to defects of the collectors, because the development of larvae was unusually scarce this year. This was evidenced by the fact that spats did not settle in large quantities on set-net or other things, which usually will be densely settled by spats in other years. Production of larvae was certainly meagre, it should be inferred. It was reported also in Saromako, Hokkaido, that collection of spats in this year was about one tenth that of ordinary year.

In 1944, collectors of type I were installed off Noheji, Yokohama and Moura and it was confirmed that collectors could stand the sea conditions during the experimental period. Production of larvae was not abundant also in this year and only 30 or 40 spats were found on shells on a rope. Until the middle of June, when the observation was made, spats could not be found by naked eyes, but in early part of July spats of 3 to 4 mm. shell length could be found and in the middle of July the largest spats measured 16 mm. in shell length. In the latter period the maximum settling of spats during the whole season was observed. When observation was made in the middle of August, the number of spats on shells was found to have decreased to about half the number observed in the middle of July, and the remaining spats were found in space between two shells and none were on the outer side of shells. All the spats were more than 10 mm. in shell length and the largest one measured 20 mm. Judging from these facts, it might be inferred that the scallop spats in Mutsu Bay attain shell length of more than 10 mm. and abandon the collector to lead bottom life about the middle of July. In Mutsu Bay, spawning begins early in March and attains the maximum in the middle of April and ends in June. The spats settle on collectors most actively in May and leave collectors in the middle of July. All these seasons just mentioned are about one month earlier respectively than those in Saromako of Hokkaido. To procure a large number of spats for experimental or breeding purposes, it is important to collect spats before the early or middle part of July, while they still remain on collectors. Collection of spats after detachment from collectors is very difficult. Settling of spats is most frequent in the depths of from one to three meters.

Artificial Production of Spats.

Natural production of spats is much influenced by climatic and oceanographical conditions, and constant yield can not be expected. To establish the scallop fisheries, therefore, it is necessary to develop a method for artificial production of desirable amounts of spats at will. When taken out of the gonad, the scallop eggs tends to disintegrate for the most part, as far as our experience proceeded, and it has been difficult to obtain ripe eggs in large quantities. One of the present authors, Kinoshita succeeded with scallops of Saromako to induce spawning and to obtain ripe eggs in large amounts by raising the temperature of the aquarium water by 5 or 6 degrees and increasing the hydrogen ion concentration by adding sodium hydroxide to the medium. With the scallop of Mutsu Bay on the contrary, we succeeded to induce spawning by keeping the scallops in water of lower temperature and lower salinity for one or two days after collection and then returning the animals to the temperature of the habitat. The scallops, when collected, seemed to have discharged ripe sexual products, and during one or two days' sojourn at lower temperature the gonad would have advanced maturation and accumulated ripe eggs and sperm, which were discharged on return to the temperature of the habitat. No necessity of increasing hydrogen ion concentration was observed. The scallop seems to discharge sexual products gradually as they ripe when the temperature rises favorably for spawning. To obtain large amounts of ripe eggs and sperm, it seems therefore convenient to keep the scallops at a lower temperature and inhibit spawning in order to accumulate the ripe eggs and sperm, and then to return to the normal temperature to induce active spawning at once. This method seems to be more favourable than the alkali treatment, as the eggs need not be washed in the former method, as is needed in the latter. By our method just mentioned large quantities of ripe eggs could be obtained, fertilised and raised for three weeks when the larvae attained the shell length of 200 microns.

Kinoshita observed in Saromako that spats with shell length of 130 microns had already settled on collector, and our larvae also would have settled if conditions had been favourable.

Transplantation of Spats Produced in Lake Saromako, Hokkaido.

Studying artificial production of scallop spats in Mutsu Bay on one hand, we attempted also to transplant spats produced in Saromako to Mutsu Bay to raise as a basis for practical fisheries. This experiment was carried out in cooperation with Aomori Prefecture. Spats were carefully detached from the collector shell by brushing, and were kept in baskets in sea water of the habitat for one or two days, then were placed in the shipping boxes, especially devised by the Fisheries Experiment Station of Hokkaido and used for several years and found quite satisfactory. On August 21, 1943, they were carried on a track from the ground to the railway station, then on a freight car to Aomori. Further transportation was made by land or on a motor boat to respective points where the spats were discharged. The whole transportation took 69 hours, the longest record of transportation period of scallop spats, and this was accomplished on August 24. The shipping boxes were double walled and the space between the outer and inner walls was filled with ice to keep the spats at low temperature; the interior of the box was divided into four layers by movable frames with fine net on the bottom to support spats, which were covered with sea weeds to keep moisture. During the transportation, ice was supplied anew several times as it melted away, and the temperature of the interior, which was 19°C. at first, gradually dropped and reached below 2° at some time, but the average was about 6°C. Six of such boxes containing 2,400,000 spats were supplied to Aomori Prefecture by the courtesy of the Fisheries Experiment Station of Hokkaido and the Fisheries Association of Saromako, and one box containing about 30,000 spats was supplied for our experimental purposes. These seven boxes were carried on a freight car of 15 ton capacity and were accompanied by two curators. The spats were discharged in the following three grounds:—

1. Off Tazawa, Kominato, Higashi Tsugaru Gun.
2. Off Noheji, Kamikita Gun.
3. Off Kawauchi, Shimokita Gun.

The box destined for our use was received at Aomori Station at 9 a.m., August 23, carried to the Marine Biological Station by land, where it arrived at 1 a.m. next morning. The temperature of the interior of the box was 11°C., and about one third of the ice remained unmelted. The box was opened and sea water was sprayed at intervals and the temperature of the box was allowed to rise gradually. At 4 a.m. the temperature rose up to 20° and the spats were taken out of the box and transferred to five table aquaria of 60 liter capacity. Each aquarium received 2,000 spats, and running sea water was circulated. Besides, three baskets with concrete bottom, 48 cm. × 63 cm. × 42 cm., covered with three different kinds of bottom materials respectively, contained 3,000 spats and were placed in natural marine reservoir, which is about one meter deep at high tide and communicates with outside sea-water. The remainder of the spats were distributed freely in the reservoir just mentioned and in the small harbour of the laboratory. When the box was opened, all the spats were found quite healthy clapping the shell, and transportation seemed quite successful. Unfortunately, however, the spats in the table aquaria, in natural reservoir in baskets, began to die from the afternoon of Aug. 24 to the morning of 25th, and the percentage of survivors was only 2% when examined on 26th. Percentage of survival of spats freely distributed in the natural reservoir and in the harbour could not be confirmed, but many corpses found suggest a high death rate. The temperature of the sea water of Saromako, when the spats were collected, was reported to be 24°C. and that at Asamushi was 25 to 26°, so that the difference was only one or two degrees. Although we have no data as regards the lethal temperature for scallop spats, it seems rather probable that the high death rate of apparently healthy spats is due to a sudden change of temperature, which the spats suffered when they were taken out of the shipping box and transferred to the sea-water of Asamushi. The temperature of the former was 20° and that of the latter was 26°C. The sea-water temperature is highest in August at Asamushi and it was unusually high in this year. If the change of temperature had been more gradual and the temperature of sea-water at Asamushi had been lower, then the spats would have survived for the most part. Transplantation experiment, therefore, does not seem to be hopeless and is desirable to be repeated.

Bottom Materials and the Spats.

It has been said by fishermen that transplantation of spats would be successful only when the spats are more than three centimeters in shell length. The spats just beginning the bottom life would be about 10 mm. in length and not very resistant against various unfavorable conditions and would be unable to develop satisfactorily. To ascertain these points, the following experiment was carried out. Three table aquaria were used, their bottom being covered each with a layer of (1) gravel, (2) mud or (3) a mixture of sand and mud, 5 cm. deep. The aquaria were circulated with running sea-water, and each received 20 healthy spats, which had been transported from Saromako and survived several days already in healthy condition. After it was observed that no spats died in any of the aquaria during the first four days of experiment, the bottom material and the spats were gently disturbed with a piece of rope. In the aquarium with gravel the water bacame turbid and re-

Table I.

Aquarium Number and Bottom Material	Oxygen Content per Litre, cc.		Survival
	Before Disturbance	After Disturbance	
(1) Gravel	4.7	4.3	20
(2) Mud	4.7	0.7	3
(3) Sand and Mud	4.7	1.0	4

turned clear in 30 minutes, but the other two aquaria took ten hours to recover to the initial state. Oxygen content per litre of the aquarium water was determined before and after disturbance. The results are as follows:—

All the spats in No. 1 were found healthy, showing no visible injury, while in No. 2 only 3 survived, two of which were found attached to the glass side wall of the aquarium and one on the bottom of mud; in No. 3, 4 survived all attaching to glass side wall. All the survivors continued to live for a long time enough, and it was confirmed that spats can grow even on muddy bottom unless it is disturbed. Disturbance of sea bottom, natural or artificial, will cause oxygen deficit and destroy various bottom organisms. Here we see clearly the evidence for importance of controlling the fisheries by trawl, for the purpose of promoting scallop fisheries.

Growth Rate of Reared Spats.

Twenty healthy spats from Saromako were contained in the basket mention-

Table II.

Shell Length at the Beginning of Exper.	At the End of Experiment		
	Shell Length	Shell Height	Shell Breadth
13.1 mm.	61.5 mm.	59.1 mm.	13.2 mm.
12.6	55.1	53.5	11.9
12.5	54.3	53.8	12.5
12.3	51.2	49.3	12.2
12.1	50.9	49.8	12.7
11.8	49.7	47.4	12.1
11.7	48.1	47.4	11.2
11.7	47.7	45.9	11.4
11.6	46.0	44.5	10.2
11.5	44.8	43.7	10.1
11.3	43.4	43.3	9.8
11.2	41.4	41.0	9.5
11.2	39.7	39.0	9.3
11.0	39.2	38.5	9.4
10.9	32.5	32.6	8.0
10.8			
10.6			
10.1			
9.6			
9.2			
Average: 11.34	Average. 47.0	Average: 45.3	Average: 10.9
Maximum: 13.1	Maximum. 61.5	Maximum: 59.1	Maximum: 13.2
Minimum: 9.2	Minimum: 32.5	Minimum: 32.6	Minimum: 8.0

ed above and were placed in the natural reservoir also mentioned above. The period of rearing ranged from August 30, 1943 to July 6, 1944, about ten months. The reservoir communicates with two openings to the exterior seawater, but the conditions in the reservoir would have been much less favourable than in the natural habitat, and the growth of the spats would have been considerably delayed. The measurements of the shell size at the beginning and at the end of the experiment are given in Table II.

In November, 1943, the shell length attained more than 30 mm. and the survival rate at the end of experiment was 15/20, 75%.

Activity of the Eggs and Sperm.

When the eggs were taken out of gonad and inseminated immediately, the fertilisation percentage was very low, 3%, and gradually increased when inseminated after a lapse of time, reaching the maximum, 23%, when inseminated after four hours. This might be, however, different in natural spawning, and further studies are needed.

Optimum Temperature for Development.

Observations were made upon the development of the fertilised eggs, kept in a differential thermostat devised by Tauchi, which has ten compartments for different temperatures and admits simultaneous experiments at different temperatures. The result shows that the favourable temperature for development of the fertilised eggs ranges from 10° to 15° C. and the optimum is 12°C.

Optimum Salinity for the Development.

Brief experiments were carried out to determine the salinity range favourable for development of the fertilised eggs, and the result shows that the optimum salinity is 3.7%. Below 3.0% and above 4.0%, the development seems almost impossible.

Conclusion.

The results of our studies on the scallop in Mutsu Bay have been briefly outlined above. Many problems remain unsolved and need further investigation. It might be said, however, that promotion of scallop fisheries on the scientific basis is very promising, judging from the results of our investigation.

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ON THE PRESENCE OF VANADIUM IN CERTAIN PACIFIC ASCIDIANS.1)

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(With 1 Text-figure)

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INTRODUCTION

The presence of vanadium in the ascidian body was first reported by Henze (1913) on the mulberry formed cell, so called "vanadocyte" in the blood of the Mediterranean species, Phallusia mammilata which is combined with the green coloured chromogen. He further described the presence of this element in other three species, Ascidia mentula, Ciona intestinalis and Diazona violacia.

Hecht (1918) noticed the presence of vanadium in the green coloured blood cells of the Atlantic ascidian, Ascidia atra.

Spectroscopically Azéma and Pied (1930) demonstrated the presence of vanadium not only in the simple solitary ascidians, Phallusia mammilata, Phallusia fumigata and Ascidia mentula, but also in the compound ascidians, Botrylloides rubrum and Botryllus schlosseri.

Cantacuzène and Tchakirian (1932) using a considerable number of the European species, determined the vanadium content in Ciona intestinalis, Ascidia mentula, Ascidia fumigata, Ascidiella aspersa, Botryllus schlosseri, Styllopsis grossularia, Polysinorata Lacazei, and Leptolinum griseum.

In 1939 Webb reexamined the vanadium content in the large number of the European species including the species so far studied, by the spectroscopic and chemical methods and demonstrated the presence of vanadium in Ascidia aspersa and Ascidia scabra but absence in Botryllus schlosseri, Botrylloides leachi, Tethyum partitum and Microcosmus sulcatus. From these findings Webb concluded that vanadium is characteristically present among the members of the primitive families, but it has been lost in the more specialized families.

Since 1933 the present writer has studied the blood tissues of the Pacific ascidians, Chelyosoma siboja Oka and Ascidia samea Oka (Kobayashi 1933, 1935, 1938 and 1940). He found that free sulfuric acid is highly accumulated in the vesicular cells of the blood and in addition a green coloured chromogen is located as a minute granule nearer its nucleus of this acid cell in these two species, the blood picture of both which indicate the close morphological resemblance.

The presence of the green chromogen

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1) Contribution from the Marine Biological Station at Asamushi, Aomoriken. No. 177.

in the vesicular cells of the blood of the Pacific ascidians appeared to the writer to be homologous to that of the vanadocyte of the blood of the European and Atlantic species, and vanadium may be also present, although the former morphologically show the difference from the vanadocyte of the latter.

On the other hand, Ohuye (1936) described the presence of the green cells in his morphological studies of the blood of the Japanese ascidians, Cynthia roretzi, Styela clava, Chelyosoma siboja, and Corella joponica.

The present investigation was undertaken to test the presence or absence of vanadium in the Pacific ascidians, Chelyosoma siboja Oka and Ascidia samea Oka and other ascidians, Styela clava Herd. and Cynthia roretzi V. Drasche.

The preliminary treatment of the materials was made at the laboratories of the Marine Biological Station at Asamushi and of the Biological Institute of Tohoku University at Sendai, and the subsequent chemical analyses were carried out at the laboratories of Hakodate College of Fisheries and at Kyoto College of Textile Fibers.

For the spectroscopic analysis, I wish to express my best thanks to Mr. Jiro Furuichi, Assistant Professor of the Physical Institute of Hokkaido University at Sapporo for his help.

MATERIAL AND METHOD

Four species of the Pacific ascidians, Ascidia samea Oka, Chelyosoma siboja Oka, Styela clava Herd. and Cynthia roretzi v. Drasche were chosen for this experiment. Of these species Ascidia samea Oka was collected at Matsushima Bay near Sendai and the other three, at Mutsu Bay, Aomori prefecture.

Each freshly collected material was cleaned by removing the adhered mud and other sessile organisms, attached on the surface of the outer test. From the samples thus treated the blood and other tissues were removed from Chelyosoma siboja Oka, the soft body and the test, from Ascidia samea Oka while the whole body was used in the other three species.

The blood of Chelyosoma siboja Oka was first introduced in a vessel from a cut on the surface of its cartilaginous test by gentle pressing of the whole animal body. Then the blood was again collected in the same vessel from the outer cartilaginous test and the soft body in order to make the loss of the blood minimum.

The soft body was separated to the following four parts: the orange coloured muscles attached to the syphon plate, the mantle and branchial sac, the gut together with the associated tissues and the blood.

The cartilaginous test in which the blood vessels are anastomosed was firstly washed with the filtered seawater and then sliced into the small pieces. The test pieces thus treated, were washed with sea-water and again with the distilled water by means of the pressing machine, for the complete removal of the blood and other tissues in the test.

The sample materials thus obtained were transferred separately into the silica crusibles and were dried at 110°C. For the determination of vanadium, 3-20 g. of each dried sample were weighed in the same crusible and ashed in the electric muffle furnace at 600°C. These ashed samples were analysed by the phosphotungustate method for vanadium by Wright and Mellon (1937) which was utilized by Webb (1939). The vanadium content was determined by the colorimetric method instead of the use of Hehner's tubes.

The spectroscopic analyses were made with the ashed samples of the whole body of Cynthia roretzi and in the dissected body parts of Chelyosoma siboja: the outer cartilaginous test and the soft body. The special care was given for obtaining the pure cartilaginous test substance as it is often contaminated with the blood and other tissues.

After scraping off the sessile organisms and other outer cartilaginous test, a milky white cartilaginous test substance is obtained. The test thus obtained was minced into small pieces and then was washed with distilled water. The blood and other tissues which are imbedded in the test were freed by the pressing machine repeatedly until the washing distilled water became clear.

For the observation of the vanadium emission spectra, the Adam-Hilger's E 1 type of quartz spectrograph was employed by using the silver electrodes which method was also utilized by Webb (1939). The arc of the ascidian ashed samples was produced by the electric current (100 Volts D.C., 5 Amperes), at the distance of 4-5 mm. between both positive and negative silver electrodes, the ashed sample being settled in a hollow at the terminal of the negative electrode

The iron and silver electrodes were also employed for the standard of the wave length calculation and for the control experiment. The plate of Fuji Photo Film Co. A 1 was used for the spectrogramm with the exposure of 40 seconds.

EXPERIMENTAL RESULTS

A. Detection and quantitative chemical analyses of vanadium in the ascidians, Ascidia samea Oka, Chelyosoma siboja Oka, Styela clava Herdman and Cynthia roretzi v. Drasche.

Vanadium was first quantitatively determined by the phosphotungustate method of Wright and Mellon (1937) on the ashed samples of the four species. The results obtained in this experiment are shown in Table 1, 2 and 3.

1. Ascidia samea Oka.

As will be seen from Table 1, vanadium is contained chiefly in the soft body amounting to 0.047 mg. and trace in the test. The vanadium content thus amounts to 0.041% for the soft body and 0.014% for the whole body, the facts

Table I. Vanadium content in Ascidia samea Oka.

	Dried sample in g.	Vanadium in mg.	%
Soft body	0.1140	0.047	0.041
Test	0.2337	Trace	—
Total in whole body	0.3477	0.047	0.014

(The figures in Table I were calculated from the samples of 52 individuals.)

Table II. Vanadium content of the dissected parts of the body in Chelyosoma siboja Oka.

	Dried sample in g.	Vanadium in mg.	%
Orange coloured muscle attached to syphon plate	0.1393	0.001	0.0007
Branchial sac and mantle	0.0979	0.038	0.039
Gut and associated tissues	1.5582	0.064	0.004
Blood.	1.0131	0.031	0.003
Total in soft body	2.8085	0.134	0.005
Test	4.4000	Trace	—
Total in whole body	7.2085	0.134	0.002

(The figures in Table II were calculated from the samples of 30 individuals.)

which may be taken as the clear evidence of the presence of vanadium.

2. Chelyosoma siboja Oka.

As will be seen in Table 2, vanadium is also present in this species. It is found highly accumulated in the gut and its associated tissues (0.064 mg.) but low in the orange coloured muscular tissues (0.001 mg.). The amount in the remaining other tissues stands between them or 0.038 mg. for the branchial sac and mantle, and 0.031 mg. for the blood. However the percentage values in these different tissues were found to be the following order in their magnitude. e.g. 0.039% for the branchial and mantle, 0.0007% for the orange coloured muscle, 0.004% for the gut and associated tissues, and 0.003% for the blood.

Only a trace of vanadium was found in the cartilaginous test as in the case of Ascidia samea Oka.

Total amount of vanadium in one individual was estimated to be 0.134 mg. which amounts to 0.005% for the soft body and 0.002% for the whole body.

Comparing the results obtained in Chelyosoma siboja with those in Ascidia samea, it will be seen that the vanadium content in the soft and whole body in Chelyosoma siboja (0.134 mg.) is much greater than in Ascidia samea. But vanadium content becomes lower when these were expressed in the percentage values (0.005% in the soft body and 0.002% in the whole body) than in Ascidia samea (0.041% in the soft body and 0.014% in the whole body) as will be seen in Table 3.

3. Styela clava Herdm. and Cynthia roretzi v. Drasche.

The present writer attempted the vanadium detection on the other two species of ascidians, Styela clava and

Table III.

Species	Vanadium % in soft body	Vanadium % in whole body
Ascidia samea Oka	0.041	0.014
Chelyosoma siboja Oka	0.005	0.002
Styela clava Herdman	0	0
Cynthia roretzi v. Drasche	0	0

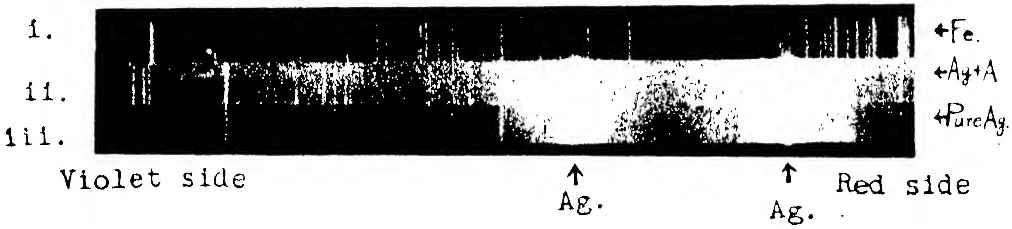
Cynthia roretzi.

On applying the phosphotungustate method of Wright and Mellon (1937) to the considerably large amount of the dried samples (11.1381 g. of Styela clava and 25.550 g. of Cynthia roretzi) of the whole body, it was found that no trace of vanadium could be detected in both species. The results obtained from both colorimetric chemical analyses of vanadium of the four Pacific ascidians are presented in Table 3.

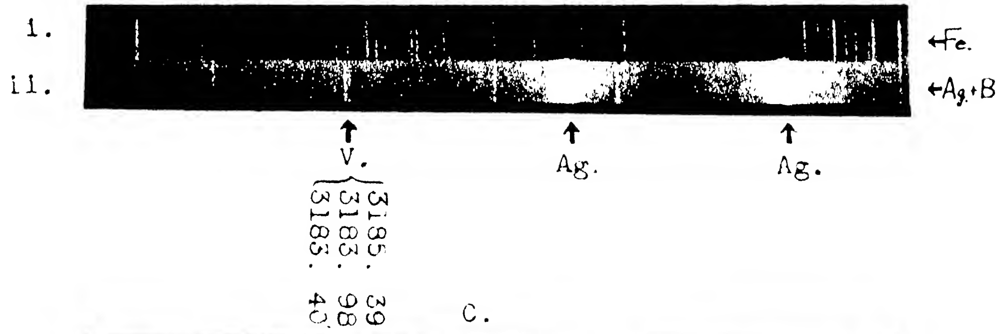
B. Spectroscopic examination of vanadium in the test and the soft body of Chelyosoma siboja Oka, and in the whole body of Cynthia roretzi v. Drasche.

The spectroscopic examination was performed with the ashed samples of the test and the remaining other soft body of Chelyosoma siboja and also of the whole body of Cynthia roretzi. As was found in the above quantitative chemical experiment, a trace of vanadium was detected in the cartilaginous tests of Ascidia samea and Chelyosoma siboja in which not only the blood vessels were intimately distributed, but both showed a close morphological resem-

A.



B.



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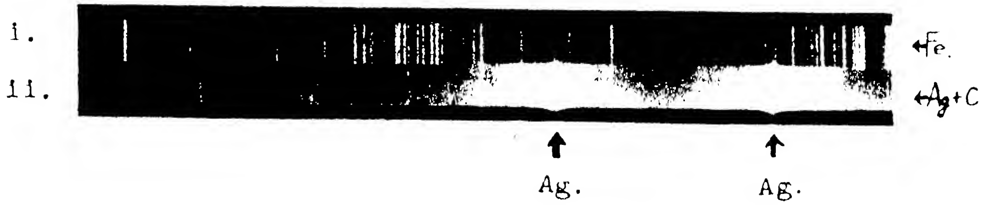

blance. (Kobayashi 1938, 1940) However, Ascidia samea and Styela clava were omitted from this experiment owing to the difficulty of obtaining the materials for this determination.

For the complete removal of the blood and other tissues from the cartilaginous test of Chelyosoma siboja, it was cut into small pieces and then was washed repeatedly with distilled water by the aid of the pressing machine.

As will be seen in Text-fig. 1, the characteristic emission spectra of vanadium (triplet of the emission lines eg. 3183.4 3184.0 and 3185.4 A) was observed in the soft body of Chelyosoma siboja but no evidence of vanadium was observed in the cartilaginous test nor in the whole body of Cynthia roretzi.

It was already shown by the chemical test that a trace of vanadium was detected in the cartilaginous test of both species of Chelyosoma siboja and Ascidia samea, but from the spectroscopical experiment I am inclined to think that its presence was due to the contamination with the blood tissues.

CONCLUSION

From the results obtained from the above experiments on four species of the Pacific ascidians, it was found that vanadium was present in Ascidia samea Oka and Chelyosoma siboja Oka, and absent in Styela clava Herdm. and Cynthia roretzi v. Drasche, although Ohuye (1936) reported the presence of the green cells in the blood of the latter two species, Styela clava Herdm and Cynthia roretzi. And it was further noticed that the amount of vanadium both in the whole and soft body was less accumulated in Chelyosoma siboja than in Ascidia samea (Table 3)

Among the isolated body tissues of Chelyosoma siboja, vanadium was absent in the outer cartilaginous test but present in the other tissues of the inner soft body in the following order

Branchial sac and mantle 0.039%, Gut and associated tissues 0.004%, Blood tissue 0.003%, Orange coloured muscle attached to the syphon plate 0.0007%.

From the above it will be seen that vanadium is present in every part of the soft body but least accumulated in the muscles attached to the syphon plate.

In Table 4 the amount of vanadium present in Chelyosoma siboja is compared with those of the European species, Ascidia mammilata (Webb 1939).

Table IV.

Species	Vanadium in blood, mg	Vanadium in whole body, mg	Blood Whole body	Author
Chelyosoma siboja	0.031	0.134	0.231	Kobayashi
Ascidia mammilata	3.75	5.46	0.687	Webb (1939)

It will be seen from the above table that vanadium in the blood and in the whole body of Chelyosoma siboja is far less accumulated than in Ascidia mammilata.

Webb (1939) arranged the percentage

values of the vanadium content of each ascidian species according to Berril's reclassification of Ascididae and showed a definite tendency of the gradual diminution, though fluctuated, from the most highly accumulated species belonging to Ascididae toward less accumulated species along the branch of Nephlocoela and Acoela or one side towards Cionidae and other side towards Pyuridae. The table in which Webb (1939) showed the relation mentioned in the above is cited in Table V.

Table V.

Family	Species	Vanadium content %	Author
Cionidae	Ciona intestinalis	0.04	Webb (1939)
Ascididae	Ascidia mammilata	0.17	〃
	Ascidia mentula	0.186	〃
	Ascidia mentula, var. rudis	0.145	〃
	Ascidia aspersa	0.145	〃
	Ascidia scabra	0.112	〃
	Ascidia samea Oka	0.014	Kobayashi
Rhodosomatidae	Chelyosoma siboja Oka	0.002	〃
Styelidae	Dendrodoa grossularia	(0.0048)	Webb (1939)
	Styela clava Herdman	0 or (trace)	Kobayashi
	Botryllus schlosseri	0 or (0.002)	Webb (1939)
	Botrylloides leachi	0 or (0.002)	〃
Pyuridae	Tethyum partitum	0 or (0.002)	〃
	Cynthia roretzi v. Drasche	0	Kobayashi
	Microcosmos sulcatus	0 or (0.002)	Webb (1939)

We notice from Webb's paper that the values given by Cantacuzène and Tchakirian (1932) appear too high when compared with that given by Webb (1939) and by the present author. The figures in the parenthesis show the uncertain or doubtful results.

When the Pacific ascidians are compared with the European species (Webb 1939), the vanadium content in the whole body of Ascidia samea and Chelyosoma siboja stands between the species belonging to Ascididae and Styelydae; that is, the values are comparable with those of Ascidia aspersa and Ascidia scabra (Ascidiella). The values of the vanadium content of these two Pacific ascidians thus appeared to indicate phylogenetically an intermediate link between the primitive type of Ascididae and the modern specialized types of Styelydae and Pyuridae.

On the other hand there exists a close morphological resemblance between the blood picture of Ascidia samea and Chelyosoma siboja, both possessing the vesicular acid cells where a minute green granule located nearer to its nucleus in the protoplasmic membrane (Kobayashi 1938, 1940), as is also found in Ascidia aspersa and Ascidia scabra (Webb 1939). However, these Pacific ascidians differ from the European species, Ascidia aspersa and Ascidia scabra, in the fact that there is no acid reaction in the test but also differ from the other ascidian species, Ascidia (Phallusia) mammilata (Henze 1913, Webb 1939) and Ascidia mentula (Henze 1939), possessing the green coloured mulberry formed cell in their blood. From the facts just given, Ascidia samea is comparable with Ascidia aspersa and Ascidia scabra (Ascidiella), and Chelyosoma siboja. The difference of the vanadium content in the blood between Ascidia mammilata (Webb 1939) and Chelyosoma siboja seems to be due to the different amount of green chromogen, accumulated in the blood cells.

SUMMARY

Vanadium content was determined in the Pacific ascidians: Ascidia samea Oka, Chelyosoma siboja Oka, Styela clava Herdm. and Cynthia roretzi v. Drasche. Vanadium was present in Ascidia samea Oka (0.014%) and Chelyosoma siboja Oka (0.002%), but absent in Styela clava Herdm. and Cynthia roretzi v. Drasche.

In Chelyosoma siboja, vanadium was extensively distributed in the blood and other associated tissues except in the outer cartilaginous test, which were less accumulated than in Ascidia mammilata (Webb 1939).

The values of the vanadium content in Ascidia samea and Chelyosoma siboja suggest an intermediate link phylogenetically between the early primitive types of Ascididae and the modern specialized types of Styelidae and Pyuridae.

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EXPLANATION OF PHOTOGRAPHS

A-C. Spectrograms of the emission spectra of the ashed samples from the body tissues of Chelyosoma siboja Oka and Cynthia roretzi v. Drasche, using the silver arc electrode. The emission spectra of the pure silver and iron arcs are shown as the standard. The emission spectra of vanadium marked as V and those of silver and iron, respectively as Ag and Fe.

A. Test of Chelyosoma siboja
  i. Iron arc.
  ii. Ash of the test of Chelyosoma siboja. Vanadium emission spectra are invisible.
  iii. Pure silver arc.

B. Soft body of Chelyosoma siboja
  i. Iron arc.
  ii. Ash of the soft body: the triplet (3183.40, 3183.98 and 3185.39 A) of vanadium emission spectra marked V is visible.

C. Whole body of Cynthia roretzi.
  i. Iron arc.
  ii. Ash of the whole body. The vanadium emission spectra are invisible.

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THE DIURNAL ACTIVITY OF A DERMESTID BEETLE, ATTAGENUS JAPONICUS.

(DIURNAL RHYTHM OF ACTIVITIES IN INSECTS AND ITS ENVIRONMENTAL CONDITIONS, NO. 13)

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(With 5 Text-figures)

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INTRODUCTION

In the previous papers, the writer had investigated the diurnal rhythm of activities in some insects (Katô, 1940a, 1940b, 1942), and it was noted that in the case of the diurnal insects whose activities depend upon their temperature reactions, the diurnal rhythm of activities must be inquired in relation to the fluctuation of the temperature factors as the outer environmental factors, and also to the variation of the body temperature as the inner physiological factors.

A Dermestid beetle, Attagenus japonicus, emerges in Sendai during the season extending from the latter part of May to the end of June, and thus we can observe a large number of them gathering on the flowers of Chrysanthemum leucanthemum. It seems to be very convenient to investigate the correlation between the activity and its environmental conditions.

Before proceeding further, the writer wishes to express his gratitude to the late Prof. Sanji Hôzawa for his continuous guidance and encouragement and also to Prof. Isao Motomura for his valuable criticisms.

THE DIURNAL RHYTHM OF ACTIVITY

The progression of the diurnal rhythm of activities found in a Dermestid beetle, Attagenus japonicus, is remarkably different from that of the Strawberry weevil (Katô, 1937); namely the maximum appearance of this beetle is observed in the forenoon, and thus it is conceivable that the diurnal rhythm of activities of this beetle may not be so simple as in the case of the Strawberry weevil. It is very interesting to notice that (Fig. 1), in the first period of emergence extending from the 28th of May to the 12th of June, the maximum flying activity is seen between the 10th hour and the 12th representing one peak-type of the diurnal rhythm of activity, but in the second period of the same extending from the 13th of June to the 28th of the same month the maximum activity is seen twice a day, the first being observed between the 8th hour and the 10th in the morning and the second between the 12th hour and the 14th, and thus represent two peaks-type of the diurnal rhythm of activity.

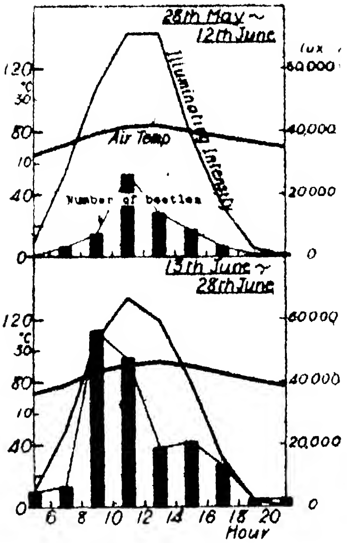

Fig. 1. The diurnal variation of flying activity of Attagenus japonicus and its environmental conditions.

THE STATISTICAL INVESTIGATION ON THE CORRELATION BETWEEN THE FLYING ACTIVITY AND THE ENVIRONMENTAL METEOROLOGICAL CONDITIONS

In order to clarify the relation existing between the general activity of the said beetle and the environmental conditions, the flying activity of this beetle was inquired in the first place.

The results obtained by the statistical investigation are represented in Table 1. From the reference to the value of correlation ratios we are able to know that a high degree of correlation exists in the case of the air temperature. And then the illuminating intensity seems to be effective only in the morning and in the evening. It is, therefore, conceiv-

Table 1. Correlation ratios calculated between the climatic factors and the flying activity of the beetle (1).

Flying Activity	Environmental Factors			
Time Interval	Illuminating Intensity	Heliothermometer	Air Temperature	Humidity
6.00— 8.00	.623±.081	.730±.062	.632±.081	.570±.089
8.00—10.00	.773±.049	.769±.050	.814±.041	.643±.071
10.00—12.00	.693±.063	.714±.059	.801±.043	.745±.054
12.00—14.00	.679±.065	.669±.067	.804±.043	.550±.084
14.00—16.00	.413±.100	.594±.078	.771±.049	.803±.043
16.00—18.00	.539±.088	.642±.072	.650±.071	.597±.082
18.00—20.00	.670±.070		.692±.066	.528±.092

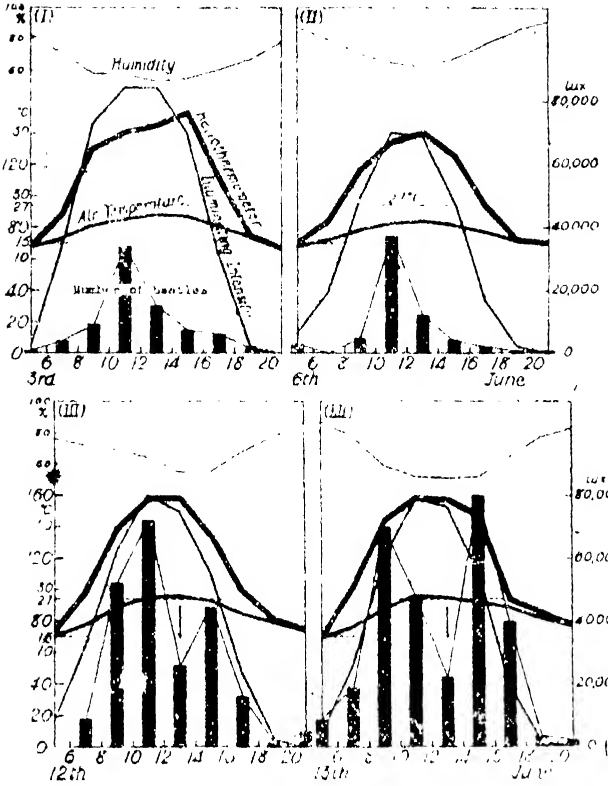

Fig. 2. Some records of the diurnal variation of the flying activity of the present Dermestid beetle.

able that, if the said activity once began, namely during the daytime, the variation in the illuminating intensity is no more important as a factor affecting upon the flying activity.

The maximum abundance of the beetle is seen earlier in the case of the two peaks-type of activity, shown in Fig. 2 (III, IV), than that in the case of the one peak-type shown in Fig. 2 (I, II). It may be understood by considering that in the latter case the environmental low temperature had inhibited the flying activity in the early morning, and this inhibiting action was removed in the former case, the air temperature becoming higher with the advance of the time of the year.

The remarkable diminution in number of beetles observed in the former case about at noon is naturally considered to be due to the inhibiting action of the high temperature, and it was observed when the air temperature reached above approximately 27°C.

It is very noticeable that the maximum environmental temperature is found before the time when the inhibiting action of the high temperature was observed (Fig. 2). The results of the statistical investigation are executed in relation to the environmental conditions and the flying activity shown for two hours after that are represented in Table 2.

Judging from the high degree of cor-

Table 2. Correlation ratios calculated between the climatic factors and the flying activity of the beetle.

Flying Activity	Environmental Factors			
Time Interval	Time Interval	Illuminating Intensity	Air Temperature	Humidity
8.00—10.00	6.00— 8.00	.779±.044	.794±.045	.487±.094
10.00—12.00	8.00—10.00	.791±.045	.738±.055	.652±.070
12.00—14.00	10.00—12.00	.703±.061	.684±.064	.698±.062
14.00—16.00	12.00—14.00	.465±.095	.701±.062	.710±.060
16.00—18.00	14.00—16.00	.597±.079	.692±.064	.604±.078
18.00—20.00	16.00—18.00	.481±.098	.644±.074	.493±.096

relation ratios, we are able to know that there exists a high grade of correlation between the activity and the environmental conditions preceding that activity.

THE GENERAL ACTIVITY AND ITS ENVIRONMENTAL CONDITIONS

We can divide the diurnal progression of activity into the following seven stages, and these were investigated in relation to the environmental conditions.

(1). **The slight movement shown in the morning:** The time when the slight crawling and the cleaning movement took place in the morning and the environmental conditions on which the above activity was started are represented in Table 3. It is permissible to think that, though the temperature environment is favourable enough to begin the activity, it is not induced if the light environment is not suitable for it. And it is also inhibited even by the strong illumination if the air temperature is low. It is estimated from Table 3 that the lowest effective air temperature is not above 13.6°C and the weakest illuminating intensity is about 1,500-2,900 lux.

Table 3. Some record concerning the time when the slight movement of the beetle took place and their environmental conditions.

Date	Time	Illuminating Intensity	Solar Energy	Heliothermometer	Air Temperature	Humidity
	h	lux	cal/cm²min	°C	°C	%
3/VI, '41	6.52	4,200~1,500		21.2	18.2	
〃	8.00	4,300~2,900		24.0	19.8	
1/VI, '42	7.00	5,300	.06	17.3	16.8	
3/VI, '42	6.30	21,500~8,600	.40~.07	17.0~14.7	13.8~13.6	62~63

(2). **Beginning of the various normal activities:** After dispersion from the resting place to the surface of the flower, such normal activities as crawling, feeding and copulation are performed. As is seen in Table 4, the normal activity begins at the reading of the black heliothermometer of approximately 20°C and at the air temperature of about 18-19°C. It seems that the illuminating intensity of about 20,000 lux or nearly so is favourable enough for the activity, and also that the light environment becomes not important as a factor influencing upon the beginning of the normal activity. It may be consequently concluded that when once the various normal activities began, the activity increases with the rising of the environmental temperature (Fig. 3).

Table 4. Some records concerning the time when the various normal activities began and their environmental conditions.

Date	Time	Illuminating Intensity	Solar Energy	Heliothermometer	Air Temperature	Humidity
	h	lux	cal/cm²min	°C	°C	%
16/VI, '41	8.30	20,000		20.7	19.2	
1/VI, '42	9.45	23,100	.313	21.2	18.9	80
3/VI, '42	8.30	16,500	.130	20.8	20.4	50
8/VI, '42	9.40	17,800	.182	19.8	18.6	87
13/VI, '42	7.30	28,500~15,200	.718~.221	21.2	19.7	80

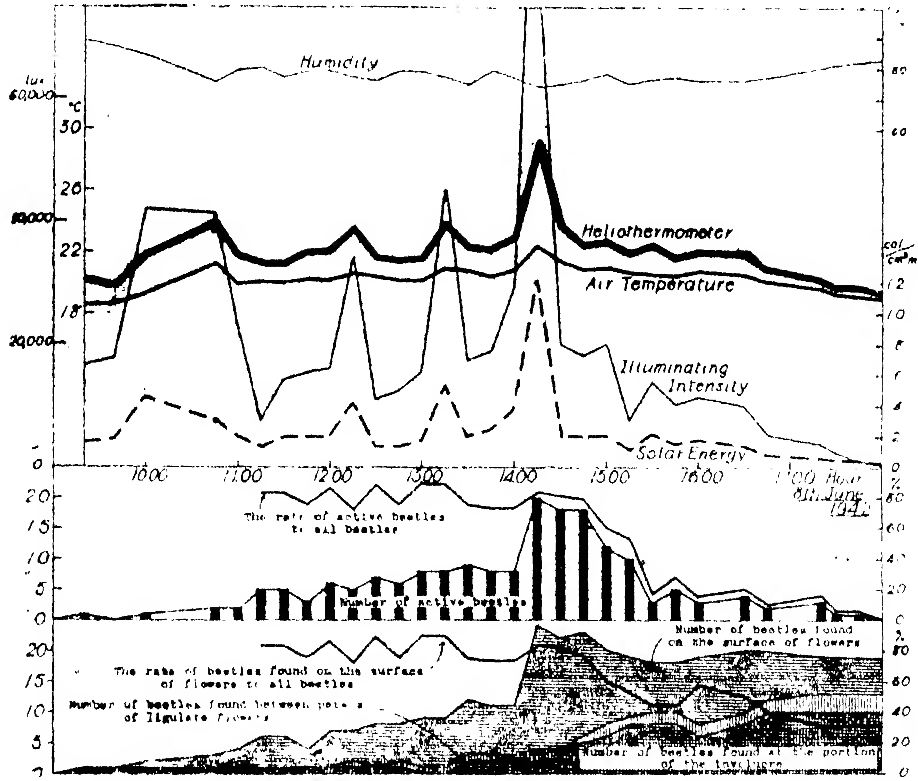

Fig. 3. The diurnal progression of the activity of Attagenus japonicus and the environmental conditions.

Table 5. Some records concerning the time when the first flying individual was observed and their environmental conditions.

Date	Time	Illuminating Intensity	Solar Energy	Heliothermometer	Air Temperature	Humidity
	h	lux	cal/cm^{2}min	°C	°C	%
3/VI, '41	8.53	6,800*		26.4	22.7	
〃	9.00	9,000*		28.5	23.3	
1/VI, '42	10.15	13,400	.175	21.0	19.1	
3/VI, '42	8.15	15,000	.129	24.7	19.4	54
8/VI, '42	9.20	16,500~14,700	.175~.153	20.2	18.5	89
15/VI, '41	9.50	24,500		19.7	14.7	

(3). **Beginning of the flying activity:** The flight begins at the same time when the normal activity takes place or rather a little earlier than that (Fig. 3). The associative action of the light and the air temperature affecting upon the said flying activity is recognized in Table 5. It seems that the minimum effective illuminating intensity which is strong enough to induce the flying activity is approximately 10.000 lux and the minimum effective temperature when the beetle are able to fly is about 15.0 C or nearly so.

(4). **Vigorous activity takes place:** After the normal activity takes place and the flying beetle begins to appear, the activity becomes vigorous.

(5). **Migration to the under surface of the flower:** When the sunshining becomes intense, the beetle represents the behavior as if they were put under the nervous condition and then migrates to the under surface of the flower, and finally all beetles gather at the portion of the involucre and become motionless. Judging from Table 6, the light intensity seems not to be the main factor effective upon the said migration. It is naturally expected that, if the environmental temperature and the reading of the black heliothermometer are respectively below 25 and 30 C, the migration of the beetle to the under surface of the flower, that is, the weakening of the activity may not be induced and the beetles may be continuously active. This fact was clearly seen in the observation made on the 8th of June (1942), when the air temperature and the reading of the black heliothermometer did not show respectively above 22°C and 28°C and the environmental temperature was always in the optimum

Table 6. Some records concerning the time when the beetle migrates to the under surface of the flower and their environmental conditions.

Date	Time	Illuminating Intensity	Solar Energy	Heliothermometer	Air Temperature	Humidity
	h	lux	cal/cm^{2}min	°C	°C	%
3/VI, '41	9.35	63,500~21,700		30.2	26.8	
16/VI, '41	11.00	56,800		29.5	24.6	
1/VI, '42	12.15	59,400	1.288	30.6	24.5	58
3/VI, '42	10.30	128,000	1.509	30.0~29.0	24.4~24.3	45
13/VI, '42	10.00	127,000~110,600	1.380~1.349	31.2	25.7	62

zone of activity (Fig. 3).

(6). **Recovering of the activity:** When the vigorous sunshining is weakened, the beetles become again active, and are found on the surface of the flower.

(7). **Entering into the resting condition:** In the dusk of the early evening the activity of the beetles become gradually weakened and finally they enter into the resting condition of the night (Fig. 3). The temperature seems not to be so effective in this case, but the light intensity seems to be the limiting factor influencing upon the activity. If the illuminating intensity becomes weaker than 10,000 lux, the activity is gradually weakened and if it becomes lower than 3,000 lux, the active beetles can hardly be seen.

It is noticeable that, differing from the case of the inhibiting action of high temperature, the beetle becomes motionless not only at the portion of the involucre but also at the border of the tubular flowers and between overlapped two petals of the ligulate flowers. The thigmotaxis of the beetles is observed in the case of the resting condition of the evening being induced from the cessation of the activity which was forced by the weak illumination. Thus it seems to be understood that the beetle which becomes motionless are found at any part of the flower.

Judging from the above mentioned fact, we learn that the diurnal rhythm of activity of the Dermestid beetle is not only governed by the temperature reaction, as was generally recognized in the case of the Strawberry weevil (Katô, 1940a) and Prumna uzume (Kato, 1942), but is also influenced by the light environment.

THE MICRO-CLIMATE OF THE FLOWER AND THE BODY TEMPERATURE OF THE BEETLE IN RELATION TO THE BEHAVIOUR OF THE SAME.

It is necessary to measure the micro-climate of the flower, for the beetle represents various peculiar behaviours on the surface of the flower and on the under surface of the flower, according to the change of their environments. Especially it is important to clarify how the involucre prepares a low temperature environment for the beetles migrated there when they were affected by the high temperature.

It is also necessary to measure the body temperature of the beetle, because the environmental temperature is effective not directly but indirectly through the body temperature upon the activity (Katô, 1940a).

The air temperature was measured by thermocouple at the seven portions, viz. (1) at the height of 10 cm above the flower, (2) at the portion of the tubular flowers, (3) on the borders of the tubular flowers, (4) on the surface of the petal of the ligulate flowers, (5) between overlapped two petals of the ligulate flowers, 6) on the under surface of the petal of the ligulate flowers and (7) at the portion of the involucre. The attention was especially paid in measuring the temperature of the thin layer of the air close to each portion of the flower mentioned above. The body temperature was measured by a specially prepared thermocouple which was used in the case of the Strawberry weevil (Katô, 1937) and which was made to measure the difference between the body temperature and the air temperature. The results of the measurements of the micro-climate and of the body temperature were represented in Fig. 4, 5.

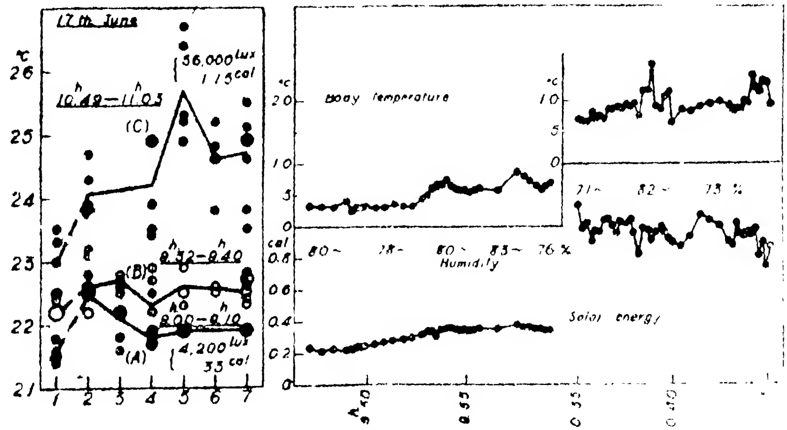

Fig. 4. The micro-climate of the flower and the body temperature of the beetle.

1: the air temperature at the height of 10 cm above the flower
2: the same on the portion of the tubular flowers
3: the same on the borders of the tubular flowers
4: the same on the surface of the petals of the ligulate flowers
5: the same between overlapped two petals of the ligulate flowers
6: the same on the under surface of the petals of the ligulate flowers
7: the same at the portion of the involucre

I. THE MICRO-CLIMATE OF THE FLOWER.

Three types of micro-climate were distinguished.

(i). The first type:— It is isothermal at each portion of the flower. This type is seen in the night and in the cloudy weather (Fig. 4, A, B; Fig. 5, B, C, D).

(ii). The second type:— The temperature is highest at the portion of the ligulate flowers (Fig. 4, C). For a while after the sun begins to shine in the morning or after breaking of clouds, the air temperature is highest either on the portion of the surface of the ligulate flowers or between overlapped two petals of the same, and it is fairly

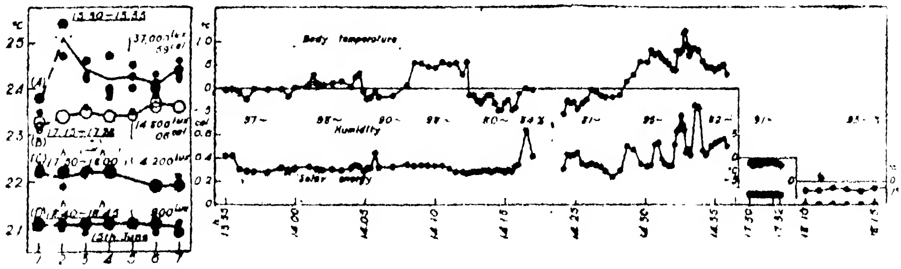

Fig. 5. The micro-climate of the flower and the body temperature of the beetle (2).

low at the portion of the tubular flowers.

(iii). The third type:— The air temperature is highest at the portion of the ligulate flowers (Fig. 5, A). This type is in general observed in the daytime under the sunshining.

II. MEASUREMENTS OF THE BODY TEMPERATURE.

The body temperature of Attagenus japonicus is remarkably affected by the solar radiant heat as is clearly seen from Fig. 4, 5. Thus the primary fluctuation process of the body temperature of the said beetle is influenced by the solar radiation. The influence of the humidity and the wind intensity (Fig. 5) must not be overlooked, but these are effective only under the condition of the weak solar radiation and are effective secondarily upon the primary process, controlled by the solar radiation. This phenomenon is just identical with the case of the Strawberry weevil. The body temperature is 1-3°C higher than the air temperature under the sunshining, and therefore the absorption of the radiant heat is similar to or a little weaker than that of the Strawberry weevil (Katô, 1937).

III. THE RELATION BETWEEN THE MICRO-CLIMATE OF THE FLOWER AND THE BEHAVIOUR OF THE BEETLE.

It may be permissible to think that the type of the micro-climate does not give any influence directly upon the behaviour of the beetle and that the temperature environment itself to which the beetle is exposed and the body temperature of the beetle itself are the factors that control the behaviour of the beetle.

The said migration of the beetle is always observed under the strong sunshining and therefore the micro-climate of the flower belongs either to the second type or to the third. Accordingly the remarkable temperature gradient is recognized at various portions of the flower, and thus the temperature at the surface of the flower is about 1°C higher than that of the under surface of the same. Moreover the body temperature of the beetle which is active on the surface of the flower is about 1-3°C higher than the air temperature, influenced by the solar radiation. Thus it may be concluded that the beetle resting at the portion of the involucre is said to be exposed to the temperature environment about 2-4°C lower than that of the surface of the flower. It is noticeable that the low temperature environment observed at the portion of the involucre is not always made only by the low air temperature, but by the absence of the solar radiation. The estimation that the body temperature in the case of the migration may be 26-27°C judging from Fig. 4, 5, is just identical with the fact that the said behaviour was observed when the air temperature and the reading of the black heliothermometer were above 25 and 30°C respectively.

CONCLUSION.

In the present paper, the writer investigated the correlation that exists between the diurnal rhythm of activity of the Dermestid beetle, Attagenus japonicus, and its environmental conditions, and he also investigated the main factors that control the said activity and the mechanism of the rhythm of activity.

In the case of the Dermestid beetle the activity takes place in the morning when the inhibiting actions of low temperature and also of the weak illumination are removed. When once the various normal activities took place, the beetle becomes more active with the rising temperature. In the dusk of the early evening the beetle enters into the resting condition, controlled by the weak illuminating intensity.

On the course of the said progression of the diurnal rhythm of activity, the weakening of the activity is observed in the daytime when the sun shines intensely and the temperature environment becomes very high.

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SCIENCE REPORTS OF THE TOHOKU UNIVERSITY, 4TH SER.
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ON HYDRAMOEBA HYDROXENA (ENTZ) DISCOVERED IN JAPAN.

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(With 2 Text-figures)

(Received September 30, 1948)

In November of 1943, Hydramoeba was first discovered on the fresh-water polyp, Hydra japonica, which was collected from a pond near Sendai. During the period from this season to the autumn of 1947, the amoebae were found several times on five species of fresh-water polyps obtained from various localities.

The amoeba with its host was fixed in Bouin's picroformol and sectioned at 5, 7 and 10 microns thick, and stained by Heidenhain's iron-alum haematoxylin.

The writer is indebted to the Late Prof. Sanji Hôzawa and to Prof. Mutsuo Katô for their kind guidances.

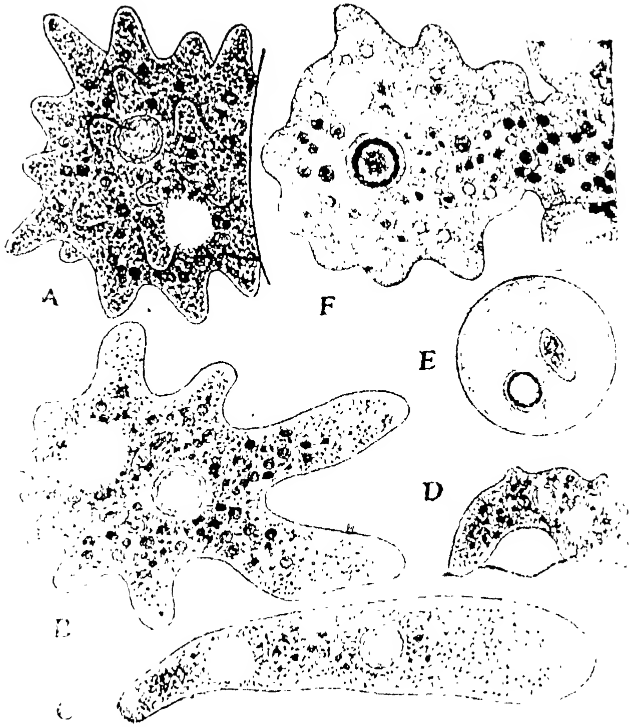
OBSERVATIONS

Form.—Very changeable. Form on the body-surface of the host (Text-fig. 1, A and D): When at rest, commonly Verrucosa-type, with knob- or finger-like pseudopods, when in motion, more elongated in shape, with an enlarged main pseudopod and a few small ones, and occasionally the animal crawls around making the so-called walking movement. Form in the coelenteron of the host (Text-fig. 1, F): Usually Proteus-type, with somewhat indistinct pseudopods, or Verrucosa-type. Form detached from the host (Text-fig. 1, B and C): Typical Proteus-type in general, but several hours after detachment changing to typical Limax-type, showing actively Planaria-like locomotion at about 178 microns per minute (observed at 20°C).

Size.—Relatively variable according to its form. 95% fiducial ranges of mean of maximum length of body are as follows: Verrucosa-type, 41.2-53.8 microns; Proteus-type, 69.4-90.0 microns; Limax-type, 136.4-156.2 microns; individuals in the coelenteron, 36.8-39.2 microns.

Protoplasm.—Distinguishable into a rather thick and transparent ectoplasm and a granular endoplasm which is coloured slightly bluish grey in Verrucosa-type and slightly greenish blue in Proteus- and Limax-types.

Nucleus. (Text-fig. 1).—Spherical or rather ellipsoidal in shape, suspended within a perinuclear vacuole, and light bluish and fairly strong light-refractive in life, 10.47-11.69 microns (95% fiducial range of mean) in long diameter, and commonly single, but sometimes 2 or 3 in number. Its structure is the so-called modified vesicular type, and almost



Text-fig. 1. Hydramoeba hydroxena from Japan.
A, Verrucosa-type. B, Proteus-type. C, Limax-type. D, Form in walking movement. E, Cyst. (All are drawn from living specimens). F, An amoeba in the coelenteron of the host, feeding on its endodermal cells. A–C, ×500; D, ×300; E, ×700; F, ×950.

agrees with those described by Reynolds and Threlkeld (1929), and ectokaryosomal chromatin 2-3 microns thick and endosome 4-5 microns in diameter (ca. 1/3 of that of nucleus).

Crystals in the endoplasm.—Many in number, of orthorhombic form, 1-2 microns in length, and disappear in the sectioned specimen.

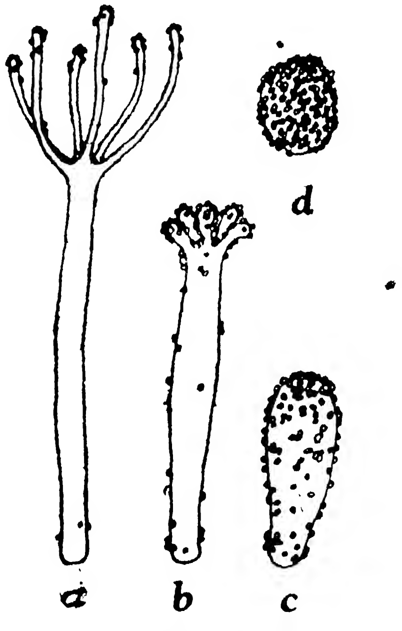
Small spherical bodies in the endoplasm.—Relatively many in number, usually located near the margin of endoplasm, 2.5-5.0 microns in diameter, strong light-refractive in life, and appear vacuoles in the sectioned specimen (Text-fig. 1, F).

Contractile vacuole.—Usually single but sometimes 2-3 in number, located generally in the posterior half of the animal, and rather larger than nucleus in maximum expansion.

Cyst. (Text-fig. 1, E).—Commonly spherical in form, 27.5-29.7 microns (95% fiducial range of mean) in diameter, surrounded by a thick membrane which shows strong light-refraction, and with single or more nucleus and sometimes provided with the nematocysts of the host and a large vacuole. The encystation is ordinarily observed soon after the death of their hosts and sometimes at the detachment from them.

Relation between the amoebae and

their hosts.—During the early stage of infection, the amoebae attach mainly to the tentacles of their hosts, especially to their tips, and thus these tentacles represent no elongated normal attitude (Text-fig. 2, a). The tentacles then become shorter and the parasites spread over the surface of the column (Text-fig. 2, b). Thus the host becomes inactive and can hardly capture its prey. Thereafter the polyp tends to the phenomenon of so-called "depression." In such condition of infection, there are also many amoebae in the coelenteron of the host, and the tentacles disappear before long (Text-fig. 2, c). In the endoplasm of these parasites, numerous food-bodies ingested from their hosts are present, and thus in the sectioned specimens of the infected polyp the amoebae are observed feeding on the cells of the polyp (Text-fig. 1, F). The nematocysts of the polyp are ingested into the endoplasm of the amoebae in undischarged state. In the late stage of infection, the column becomes rather a small ellipsoidal or spherical form, with numerous amoebae attached to the whole surface and in its coelenteron (Text-fig. 2, d), but soon after vanishes from sight. Death of the host may occur in about one week after infection in the case of the laboratory observation (cultured at ca. 25°C).



Text-fig. 2. Several stages of the infected fresh-water polyp with amoebae. ca. × 10.

Several species of the fresh-water polyp in Japan were infected with Hydramoeba and the parasites were almost similarly pathogenic to each species. But the resistance to the parasites appears to be rather strong in the case of Pelmatohydra robusta, a species of large form, and also in the polyps symbiosed with the green algae.

The amoebae have been hitherto observed in following seasons: November (in nature, a pond near Sendai) and December (in laboratory) 1943; April, July, and August (in laboratory) 1944; June (in nature, a reservoir in Miyato-shima, Matsushima Bay, Miyagi Pref.) and July (in laboratory) 1945; September (in nature, a pond near Kogota Miyagi Pref.). Thus the amoebae seem to appear in all seasons of the year except for the mid-winter, and no marked seasonal variation in form and habit of the amoebae was observed.

Some experiments were executed on the effect of the hydrogen ion concentration, temperature and salinity upon the amoebae which are infecting on the two species of polyp, Pelmatohydra robusta and Hydra magnipapillata. Each of 10 polyps in the early, middle and late stages of infection which were cultured in the medium of pH 7.0 were transferred the culture media of various hydrogen ion concentrations prepared to be in the range from pH 5.0 to 9.4 at interval of 0.4 (cultured at 22°C). Conclusively, a remarkable difference of the infecting progress was not recognized between the experimental media and the original one. Uninfected polyps which were put in the culture media mentioned above, except for those of pH 5.0, 5.4, 9.0 and 9.4, were in normal healthy condition. Sudden changes of temperature in the range of torelance for the polyps produced no effect on the pathogenicity of the amoebae. Thus, it seems that such changes of the environmental conditions above alluded to give no special effect on the pathogenic activity of the parasites. However, the diluted sea-water in the range of torelance for the polyps was effective to the destruction of the amoebae. For instance, each of 20 infected polyps in the three stages mentioned above were placed in diluted sea-water of 0.3% (cultured at 22°C). 24 hours after transferring, 62% of the polyps were set free from the infection of the amoebae and there gradually recovered their health. In this case, however, the host in the late stage of infection almost died, and it appears to be remarkably unfavourable for the polyps to be put in more diluted sea-water.

DISCUSSION

The present Hydramoeba resembles the European (Entz, 1912; Wermel, 1925) and North American Hydramoeba (Reynolds and Looper, 1928; Reynolds and Threlkeld, 1929; Threlkeld and Reynolds, 1929) in its structure and habit. But these amoebae recorded from the above-mentioned three localities differ from each other in some features as shown in the following Table I.

The Japanese Hydramoeba is smallest in size among them and it differs from the European amoeba in several characters and is rather closely allied to the American form in general, especially in the pathogenicity to its host.

Table I. Comparison between features of Hydramoeba described from three localities.

	European amoeba	American amoeba	Present amoeba
Length of body	100—380 μ	60—380 μ usually 125—150 μ	26—210 μ, 95% fiducial range of mean: Verrucosa-type, 41—54 μ Proteus-type, 69—90 μ Limax-type, 136—156 μ
Number of nucleus	1—5, usually 2	1—4, usually 1	1—3, usually 1
Long diameter of nucleus	14—30 μ	10—18 μ	10—16 μ, 95% fiducial range of mean: 10.5—11.7 μ
Length of crystal	3—4 μ	——	1—2 μ
Diameter of spherical body	9—12 μ	——	2.5—6.0 μ
Pathogenicity	None	Serious	Serious

Reynolds and Threlkeld clarified the morphological correspondence between the European and American amoebae by the exchange of both specimens. And as to the physiological difference between both amoebae, Threlkeld and Reynolds considered that it is due to the difference of the hydrogen ion concentration in the culture medium. But this conclusion appears not to agree with the result of the writer's experiments in which the materials of the host was Pelmatohydra robusta, a species distinct from the American Pelmatohydra oligactis. It was concluded in the present study that the pathogenic activity of the parasites suffers no marked influence by the difference of the hydrogen ion concentation in the culture medium.

Here, however, judging from the close resemblance in the general characteristics of these amoebae and the descriptions by Entz, Reynolds and other American authors, the present Hydramoeba in Japan should be identified as Hydramoeba hydroxena (Entz).

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ON THE MORPHOLOGICAL VARIATION OF THE NEMATOCYSTS IN PELMATOHYDRA ROBUSTA ITÔ.

(STUDIES ON THE NEMATOCYSTS OF THE FRESH-WATER POLYP. NO.I)

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(With 4 Text-figures)

(Received September 30, 1948)

The morphology of nematocysts in the fresh-water polyp has been considered as an important taxonomic feature. Since the morphological stability of the nematocysts is not known, the writer made a statistical investigation on the problem with the results herein stated.

The writer wishes to thank Prof. Mutsuo Katô for his supervision during the course of study.

MATERIALS AND METHODS

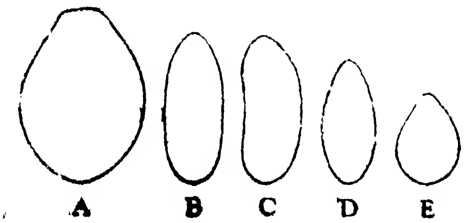
The 70 individuals of Pelmatohydra robusta used for the investigation were taken from a large jar placed in the yard of the Biological Institute. These fresh-water polyps are descendants from the individuals which were collected from a spring at the Yokoyama-Fudôson, Miyagi Pref. in 1944 and kept under natural conditions in the above-mentioned jar. The measurements were made on 5962 nematocysts during November and December of 1947. The polyps taken at random from the large jar were immediately enclosed in phenol-glycerin (distilled water 200 cc, glycerin 200 cc, and crystalline phenol 1 gr.) and the length and width of the nematocysts found in them were measured by the ocular micrometer. In the case of comparison between the nematocysts in the parent and those in the bud, the budded individuals were cut into a parent-body and a bud-body, and separately prepared then examined as mentioned above.

RESULTS AND DISCUSSION

I. Morphological variation of the nematocysts

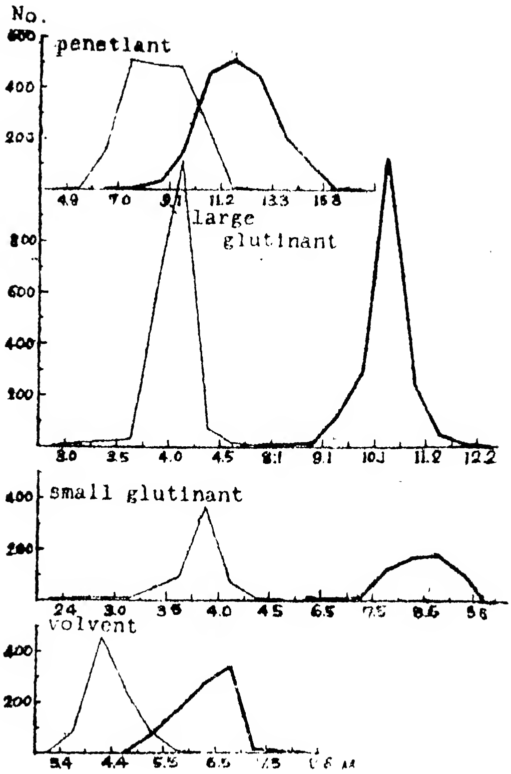
There are four types of nematocysts in the fresh-water polyp, viz. penetrant, large (streptoline) glutinant, small (stereoline) glutinant, and volvent; their forms are shown in Text-fig. 1.

The frequency polygons obtained from the measurements of length and width of the nematocysts of the four



Text-fig. 1. Four types of nematocysts in Pelmatohydra robusta.

A, Penetrant. B and C, Large glutinants. D, Small glutinant. E, Volvent. ×2000.



Text-fig. 2. Frequency polygons of length and width of nematocysts of four types.

Polygons of fine line, width; those of bold line, length.

types appear to represent respectively the normal frequency distribution (Text-fig. 2).

The means and coefficients of variation of length and width of the nematocysts of the four types are given in Table I. The significant differences between the means of size of the four types are respectively recognized, and the mean value decreases in the orders: penetrant, large glutinant, small glutinant and volvent, in length, penetrant, volvent, large glutinant and small glutinant, in width. The coefficient of variation is considerably small in general, varying between 4 and 10 per cent. The difference between them are individually significant, and thus the variability of length and width is largest in penetrant and smallest in large glutinant. Generally that of the pear-shaped nematocysts of two kinds is larger than that of large and small glutinants.

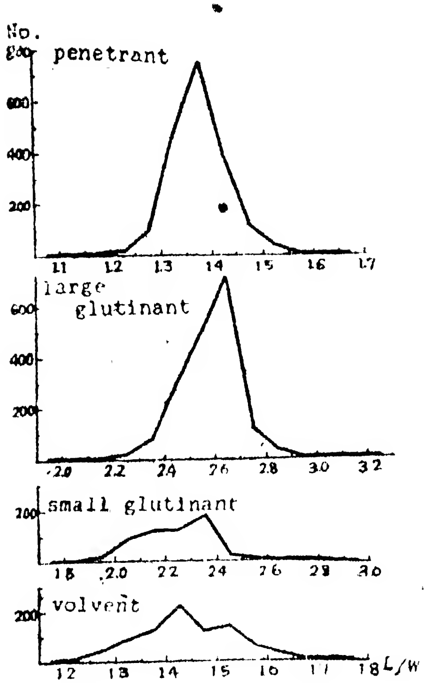
Table I. Means and coefficients of variation of length and width of nematocysts of the four types.

	Mean (μ)		Coefficient of variation (%)		Number of individuals
	Length	Width	Length	Width	
Penetrant	11.92±0.03	8.73 ±0.02	9.40±0.15	9.93±0.16	1899
Large glutinant	10.34±0.01	3.997±0.004	4.05±0.07	3.82±0.06	1835
Small glutinant	8.52±0.02	3.80 ±0.01	6.35±0.19	5.53±0.16	577
Volvent	6.25±0.02	4.34 ±0.01	7.74±0.19	9.30±0.22	851

The frequency polygons drawn from the values of L/W (L=length, W=width) of the nematocysts of the four types (Text-fig. 3) seem to show respectively the normal frequency distribution.

The difference between the means and between the coefficients of variation of L/W of the four types are individually significant. The mean increases in the order: penetrant, volvent, small and large glutinants. The coefficient of variation of L/W varies between 4 and 6 per cent. The variability of L/W is smaller in penetrant and volvent, especially in the former, than that of length and width mentioned above, but rather larger in large glutinant. And it is smallest in penetrant and next in large glutinant (Table II).

Judging from the coefficient of correlation between length and width, a positive correlation is recognized in each type of nematocysts, being highest in penetrant and lowest in large glutinant. It increases in the reverse order to that of the mean of L/W (Table II).



Text-fig. 3. Frequency polygons of L/W of nematocysts of the four types.

Table II. Means and coefficients of variation of L/W, and coefficients of correlation between length and width of nematocysts of the four types.

	Mean	Coefficient of Variation	Coefficient of correlation
Penetrant	1.377+0.001	3.78±0.06	0.924±0.003
Large glutinant	2.590±0.003	4.62±0.08	0.296±0.021
Small glutinant	2.238±0.006	6.04±0.18	0.523±0.030
Volvent	1.443±0.003	6.27±0.15	0.723±0.016

Therefore, judging from the statistical analyses on the measurements of nematocysts as stated above, the following conclusions may be reached. The variability of length, width and L/W of the nematocysts of the four types is rather small, namely the morphological stability of the nematocysts is considerably high. Among the four types of nematocysts, the large glutinant, is smallest in the variability of length and width and also not too large in that of L/W though the length does not highly correlate with the width. These characters of the large glutinant, together with the character of its thread, seem to be valuable as a taxonomic characteristic. Although the penetrant is largest in the variability of length and width, it is smallest in that of L/W and shows the highest positive correlation between length and width.

In consequence, the large glutinant is most stable in length and width, namely it appears to have the most stable size, while the form of the penetrant seems to be most stable, among the four types of nematocysts.

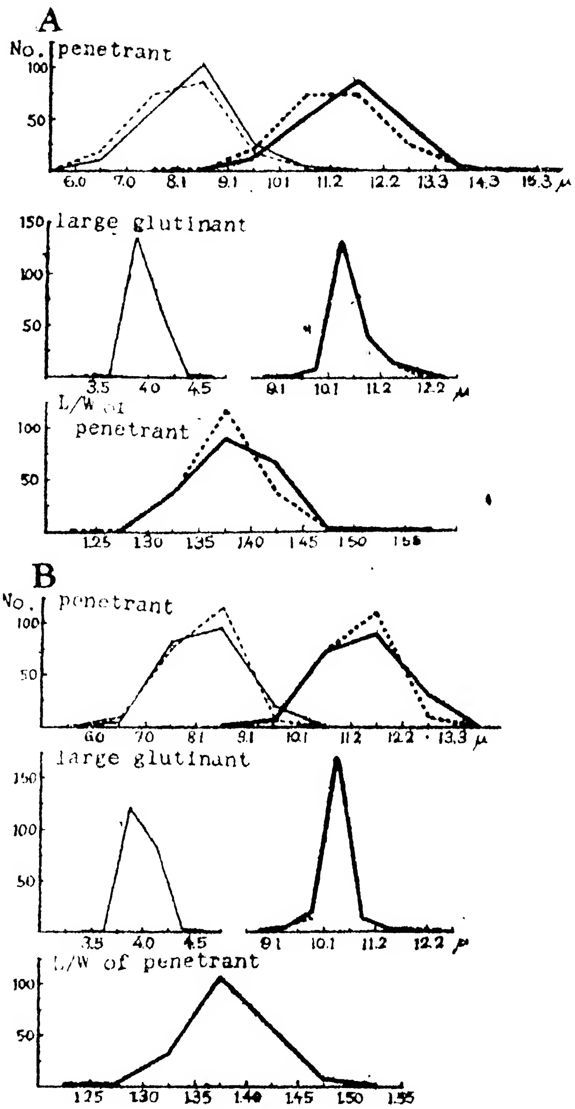
II. Morphological comparison between the nematocysts in parent and those in bud.

The measurements of the nematocysts in the buds, in the two stages of development, viz. one stage in which the tuberculose tentacles have slightly rised and the other which has just detached from the parent (the former is represented by B^1 and the latter by B^2). were compared with those of their parents (P^1 and P^2). In this case, the size of the most variable penetrant and the most stable large glutinant, and the L/W of penetrant that is most stable, as mentioned above, were selected.

The frequenecy polygons drawn from the measurements are shown in Text-fig. 4, A and B.

As regards the means of width and L/W of penetrant and length and width of large glutinant, the difference between B^1 and P^1 is not statistically significant, while the difference between the means of length of penetrant in B^1 and P^1 is barely significant. The means of all the said values in B^2 are not individually significantly different from those of P^2 (Table III).

Judging from the results mentioned above, it is easily recognized that in the course of the growing of the buds, the size and form of the said nematocysts may be not significantly different from those of their parents.



Text-fig. 4. Frequency polygons of length and width of penetrant and large glutinant and of L/W of penetrant in B_1, B_2, P_1 and P_2.

A. Comparison between B_1 and P_1.
B. Comparison between B_2 and P_2.

Polygons of fine line, width; those of bold line, length; those of broken line, bud; those of full line, parent.

Consequently, it is considered that the afore-said relations between the

Table III. Comparison between means of length, width and L/W of nematocysts in parent and bud.

	Penetrant		Large glutinant		Penetrant	Number of individuals
	Length (μ)	Width (μ)	Length (μ)	Width (μ)	L/W	
P_1	11.57±0.07	8.37±0.05	10.64±0.03	3.97±0.01	1.384±0.002	200
B_1	11.26±0.07	8.20±0.06	10.61±0.02	3.97±0.01	1.376±0.002	200
D	0.31±0.10	0.17±0.08	0.03±0.04	0.00±0.01	0.008±0.003	
R	3.10	2.12	0.75	0.00	2.67	
P_2	11.46±0.06	8.29±0.05	10.39±0.02	3.94±0.01	1.383±0.002	200
B_2	11.31±0.05	8.17±0.04	10.42±0.02	3.94±0.01	1.383±0.002	200
D	0.15±0.08	0.12±0.06	0.03±0.03	0.00±0.01	0.000±0.003	
R	1.88	2.00	1.00	0.00	0.00	

D, Difference between means. R, Difference between means / Standard error of difference.

nematocysts in the bud and parent are closely connected with the condition of production and consumption of the nematocysts in both bodies.

In conclusion, there are recognized almost no significant differences between the size and form of nematocysts in the bud and parent, and it may be noted that in the course of taxonomic study it is not necessary to take into consideration the biometrical characters of the nematocysts regarding the age of the asexually grown polyps.

In the present study the nematocysts of the sexually grown young polyps were not dealt with, because such young individuals are only seldom found in nature.

SUMMARY

1. The morphological variation of the nematocysts of the four types in a fresh-water polyp, Pelmatohydra robusta Itô, was investigated statistically and a morphological comparison between the nematocysts in the bud and in the parent was made.

2. The coefficient of variation of length and width of the nematocysts of the four types is 4-10% and that of L/W is 4-6%. The morphological stability of the nematocysts seems to be considerably high in general.

3. The variability increases in the following order: large glutinant, small glutinant, volvent and penetrant, in length and width; penetrant, large glutinant, small glutinant and volvent, in L/W. Namely the size seems to be most stable in the large glutinant and the form is in the penetrant.

4. The biometrical differences of size and form are hardly recognized between the buds and their parents.

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SCIENCE REPORTS OF THE TOHOKU UNIVERSITY, 4TH SER.
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MORPHOLOGICAL COMPARISON BETWEEN THE NEMATOCYSTS IN THE TENTACLES AND COLUMN OF PELMATOHYDRA ROBUSTA ITÔ.

(STUDIES ON THE NEMATOCYSTS OF THE FRESH-WATER POLYP. NO. II)

BY
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(With 1 Text-figure)

(Received September 30, 1948)

In a taxonomic study of the fresh-water polyp it is important whether the nematocysts are morphologically different, between the tentacles and the column. The present paper deals with the statistical investigation on this problem and on the relative abundancy of the nematocysts of the four types found in the polyp; and the observation where the nematocysts are formed or discharged was made.

The writer is indebted to Prof. Mutsuo Katô for the help rendered him.

MATERIALS AND METHODS

The 30 individuals of Pelmatohydra robusta taken from the large jar, as mentioned in the previous paper (1949), were used. These living specimens were immediately cut into two parts, viz. the tentacles and column, in stretched state, and both parts were enclosed separately in phenol-glycerin, and then the undischarged nematocysts in each part were measured by the ocular micrometer. The measurements were made in November of 1947 for the 1899 penetrants, 1835 large glutinants and 1111 volvents. All of the nematocysts of the four types which appeared in the sight of the microscope were counted and the body-part where the nematocysts are formed or discharged was observed.

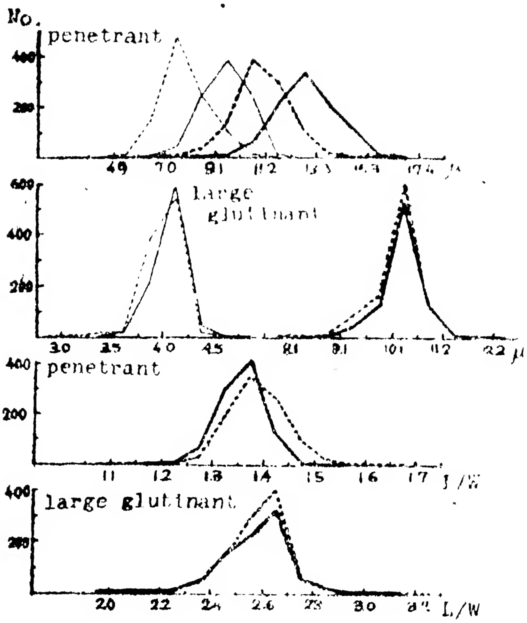
RESULTS AND DISCUSSION

I. Morphological comparison between nematocysts in tentacles and those in column.

The penetrant and large glutinant among the four types of nematocysts were examined, because the small glutinant and volvent appear to be mostly still immature in the column.

The frequency polygons drawn from the values of length, width and L/W (L=length, W=width) of the penetrant and large glutinant in the tentacles and column are shown in Text-fig. 1.

The means of length and width of the penetrant and large glutinant are significantly different between both of the



Text-fig. 1. Frequency polygons of length, width and L/W of penetrant and large glutinant in tentacles and column.

Polygons of fine line, width, those of bold line, length; those of full line, tentacles; those of broken line, column.

body-parts, that is to say, the penetrant and large glutinant in the tentacles are larger in size than those in the column, and this difference is more remarkable in the penetrant than in the large glutinant. The difference between both parts of the penetrant is more distinct in the width than in the length, but in the case of large glutinant this relation is just the reverse (Table I).

In the coefficients of variation of the above-mentioned characters, the significant difference between the tentacles and column is recognized only in the penetrant. The variability in size of the penetrant is larger in the column than in the tentacles, and this difference is relatively more distinct in the width than in the length (Table I).

Table I. Comparison between means and coefficients of variation of length and width of penetrant and large glutinant in tentacles and column.

		Mean (μ)		Coefficient of variation (%)		Number of individuals
		Length	Width	Length	Width	
Penetrant	T	12.85±0.04	9.56±0.03	8.75±0.20	8.85±0.21	922
	C	11.05±0.04	7.95±0.03	10.18±0.23	11.14±0.25	977
	D	1.80±0.06	1.61±0.04	1.43±0.30	2.29±0.33	
	R	30.0	40.2	4.8	6.9	
Large glutinant	T	10.40±0.01	4.03±0.01	3.94±0.10	3.75±0.09	858
	C	10.29±0.01	3.97±0.01	4.07±0.09	4.00±0.09	977
	D	0.11±0.01	0.06±0.01	0.13±0.13	0.25±0.13	
	R	11.0	6.0	1.0	1.9	

T, Tentacles; C, column; D, difference between means; R, difference between means /standard error of difference.

The means and coefficients of variation of the L/W of nematocysts of the two types in the tentacles and column are given in Table II. The difference between the means in the tentacles and column is significant only in the penetrant, and barely insignificant in the large glutinant. The L/W of penetrant is smaller in the tentacles than in the column, namely the individuals in the former are broader in form than those in the latter. In the coefficients of variation of L/W, the difference between the both body-parts is significant only in the penetrant similarly to the case of the mean value. And the variability of L/W of the penetrant is larger in the column than in the tentacles.

Table II. Comparison between means and coefficients of variation of L/W of penetrant and large glutinant in tentacles and column.

		Mean	Coefficient of variation (%)
Penetrant	T	1.361±0.001	2.94±0.07
	C	1.392±0.002	4.31±0.10
	D	0.031±0.002	1.37±0.12
	R	15.5	11.4
Large glutinant	T	2.581±0.004	4.74±0.11
	C	2.597±0.004	4.49±0.10
	D	0.016±0.006	0.25±0.15
	R	2.7	1.7

As mentioned above, the differences between the means of length and width of the penetrant and large glutinant in the tentacles and those in the column are statistically significant, namely the nematocysts of both types in the tentacles appear to be larger in size than those in the column. In the coefficients of variation, the difference between the both body-parts is only significant in the penetrant, and its variability is larger in the column than in the tentacles. Only in the penetrant, the means and coefficients of variation of L/W in the column are significantly different from those in the tentacles, and the individuals in the column are narrower and more variable in form than those in the tentacles.

These afore-said relations between the nematocysts in the tentacles and those in the column seem to be closely connected with the forming and discharging of them as mentioned below.

Consequently, for taxonomic purpose the differences in the size and form between the nematocysts in the tentacles and those in the column should be given detail attention.

II. Relative abundance of the four types of nematocysts found in the polyp and body-part where nematocysts are formed or discharged.

The relative abundance of the four types of nematocysts found in various portions of the polyp was represented by the percentage of the total number of nematocysts, as shown in Table III. The number of the nematocysts of the four types in the column increases in the order: small glutinant, volvent, large glutinant and penetrant. The small glutinants are extremely few, amounting to only about 1/100 of the total number and they are mostly immature. The penetrants are somewhat more abundant in number than the large glutinants, showing about more than 2/5 of the total number, and the

Table III. Means of relative abundance (represented by percentage of number) of the four types of nematocysts in column, tentacles and whole body.

	95 % fiducial range of mean (%)			
	Penetrant	Large glutinant	Small glutinant	Volvent
Column	39.79—49.39	33.25—42.25	0.61—1.31	13.55—19.85
Tentacles	9.69—11.75	4.17—6.25	3.72—6.82	76.90—80.72
Whole body	14.90—17.68	8.92—13.08	3.29—5.77	65.06—71.30

volvents which are mostly immature amount to about half the number of the large glutinants.

The number of the nematocysts in the tentacles decreases in the order: volvent, penetrant, and two types of glutinants. The volvents amount to as much as about 4/5 of the total number, thus differing from the case in the column. The penetrants are about 1/10 of the total number, and the each of the glutinants of the two types amount to only about ½ of that of the penetrant.

In the whole body, the volvents are found to exceed 3/5 of the total number, while the small glutinants amount to about 1/20 of the same. Although the number of penetrants is relatively greater than that of the large glutinants, they are less than 1/5 of the total number.

Therefore, the approximate ratios in number of the four types of mature nematocysts in the two body-parts and whole body are: penetrant: large glutinant: small glutinant: volvent=1: 1: 0: 0, in the column; 2: 1: 1: 16, in the tentacles, 3: 2: 1: 12, in the whole body. And the number of penetrant appears to be equal to the sum of those of the large and small glutinants, in the tentacles and column. Thus, if the number of penetrant is given by 1, the above ratios become: penetrant: glutinant: volvent=1: 1: 0, in the column; 1: 1: 8, in the tentacles; 1: 1: 4, in the whole body.

In the tentacles, the four types of mature nematocysts are densely arranged forming many clusters. On the other hand, in the column the germ regions wherein the nematocysts are formed are seen dispersing mostly over the body (zooid) and slightly over the stalk, and therein mainly the immature nematocysts are found in groups; and the mature penetrants and large glutinants lie scattered in the whole column. There is no germ region in the tentacles, and thus the immature nematocysts are not found. The small glutinants and volvents found in the column are mostly in an immature state; all of them may be transported to the tentacles to be discharged. The penetrants and large glutinants are also formed in the germ regions of the column, and some of them may be similarly transported to the tentacles, but the others remain in the column and are discharged there.

The biometry concerning the volvent is given below in the relation to the forming and discharging. The means and coefficients of variation of length and width, and the means of L/W, were compared between the tentacles and column (Table IV). The above-men-

Table IV. Comparison between means and coefficients of variation of length and width, and between means of L/W, of volvent in tentacles and column.

	Mean (μ)		Coefficient of variation (%)		Mean	Number of individuals
	Length	Width	Length	Width	L/W	
T	6.25±0.02	4.34±0.01	7.71±0.19	9.30±0.22	1.443±0.003	851
C	5.33±0.02	3.87±0.02	6.22±0.27	6.15±0.27	1.380±0.005	260
D	0.92±0.03	0.47±0.02	1.52±0.33	3.15±0.35	0.063±0.006	
R	30.7	23.5	4.6	9.0	10.5	

tioned values of the volvent in the column were significantly different from those in the tentacles. Thus the size of the volvent in the column was smaller than in the tentacles, but the coefficients of variation was rather larger in the latter than in the former.

Namely, the variability in the length and width of the volvent is larger in the tentacles than in the column, therefore the volvent may be enlarged in size not only in the column, but also in the tentacles. However, its form seems to be narrower in the tentacles than in the column.

In short, the relative numbers of the four types of nematocysts in the column, tentacles and whole body seem to be rather constant, and thus a remarkable difference is recognized between the tentacles and column. The mature volvents are found most numerously in the tentacles, being about four times the sum of the three other types, but are almost absent in the column. The penetrants and the glutinants of the two types show nearly the same number in both the tentacles and column. These facts are considered to be closely connected with the function of the nematocysts and also with the forming and discharging of them. The four types of nematocysts are formed in the germ regions found in the column, and all types of them are discharged in the tentacles, while the penetrant and large glutinant also discharge in the column.

SUMMARY

1. The morphological comparison between the nematocysts in the tentacles and column of Pelmatohydra robusta Itô was made statistically. The relative abundance of the nematocysts of the four types in the column, tentacles and whole body, and the body-part where the nematocysts are formed or discharged, were investigated.

2. The size of the penetrant and large glutinant are larger in the tentacles than in the column. The form and the variability of size and form of the penetrant are narrower and larger in the column than in the tentacles, but those of the large glutinant show no such differences between the body-parts.

3. The approximate ratios between the numbers of mature nematocysts of the four types are: penetrant: large glutinant: small glutinant: volvent = 1: 1: 0: 0, in the column; 2: 1: 1: 16, in the tentacles; 3: 2: 1: 12, in the whole

body.

4. The four types of nematocysts are formed in the germ regions found in the column, and all types of them are discharged in the tentacles, while the penetrant and large glutinant are also discharged in the column.

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ON SOME ENTOPROCTA FROM JAPAN.

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(With 6 Text-figures)

(Received September 30, 1948)

Up to the present time the Entoprocta of Japan has not been fully studied. In 1945, the writer has reported 4 species of Bryozoa found in the brackish-water region of Matsushima Bay, viz. Victorella pavida Kent, Bowerbankia caudata Hincks, Electra crustulenta Pallas and Barentsia benedeni (Foettinger). At that time he has also alluded to the forms of Entoprocta of the same bay saying that they are represented by seven species at least.

During the progress of the study of these animals, the writer has noticed that the species of Entoprocta obtained in Matsushima Bay should be compared with those came from the Pacific coast, by the reason that there may be seen some morphological variations among the forms found in the bay.

The writer, therefore, tried to collect the forms of this group of animals from the Pacific coast of Miyagi Prefecture, and was able to obtain following five species.

1. Loxosoma shizugawaense, n. sp.
2. Pedicellina cernua (Pallas)
3. Barentsia discreta (Busk)
4. Barentsia laxa Kirkpatrick
5. Barentsia hôzawai, n. sp.

Of these forms, Barentsia hôzawai, n. sp. was collected by the late Prof. Hôzawa from Izushima in Onagawa Bay, and was kindly forwarded to the writer for identification. The remaining forms were taken by the writer.

The present report deals with those specimens obtained from the Pacific coast of Miyagi Prefecture during 1941-1945.

Before proceeding further, the writer would like to express his hearty thanks to the late Prof. Sanji Hôzawa who has kindly helped the writer in many ways during the course of the present study. Acknowledgements are also due to Prof. Mutsuo Katô for his helpful advice and criticisms.

Description of Forms

Order Pedicellineae

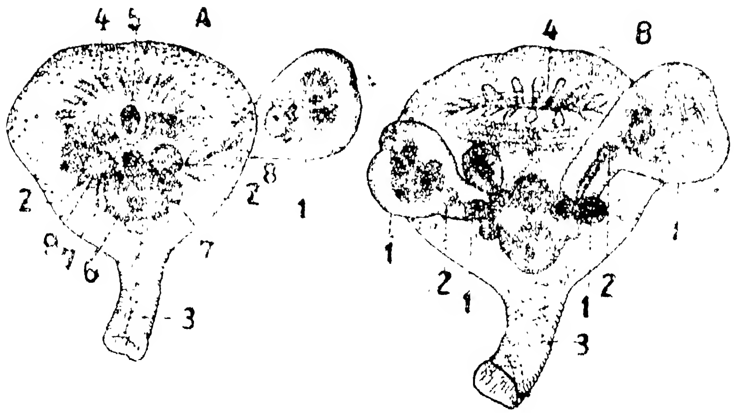
Family Loxosomatidae

1. Loxosoma shizugawaense n. sp.

(Text-fig. 1)

Size relatively large. Entire animal is about 1.5 mm. in length and is 1 mm. in breadth. Calyx rather broad being somewhat in the shape of an inverted triangle. It is flattened dorso-ventrally perhaps from the reason that it was fixed in formalin without using narcotics.

Tentacles are usually 18 in number



Text-fig. 1. Loxosoma shizugawaense n. sp.

A. dorsal view ×25

1. bud 2. sense organ 3. straight muscle 4. tentacle
5. rectum 6. stomach 7. lateral lobe of stomach
8. intestine 9. gonad

B. ventral view ×25

1. bud 2. foot-gland of bud 3. oblique muscle 4. tentacle

but sometimes are 19-20. Owing to the contracted condition of specimens, the details of the lophophore can not be stated definitely.

Stalk cylindrical, from one-half to two-thirds as long as the calyx (but rarely equally long) in the contracted state. It is wrinkled transversely on the surface. Straight muscles are present attached to the inner surface of dorsal wall of the stalk and the oblique muscles are found attached to the inner surface of ventral wall of the same.

Buds are produced as many as five at a time, (two or three of the same being found on each side). In most cases they are formed alternately on each side of the body, younger bud usually occurring at the base of nearly matured one. Stomach is provided with well-developed lateral lobes. Foot-gland present in the buds, but not in the adult.

The living specimens and living larvae have not yet been observed. The present specimens which were taken from Shizugawa Bay were found attached to the basal part of some grown-algae (Laminaria) grown in shallow water of about 1 meter deep. Previous knowledge of occurrence of any Loxosoma species in Japan is unknown. This group seems to be rather uncommon in the Pacific coast of Japan.

Family Pedicellinidae

2. Pedicellina cernua (Pallas)

(Text-fig. 2)

Synonyms

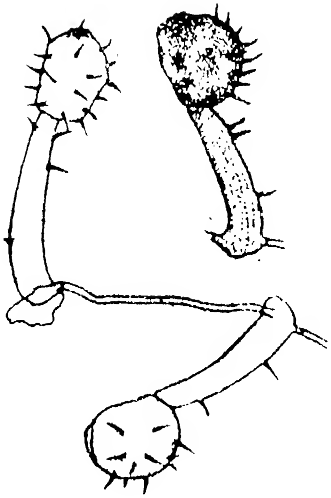
Brachionus cernuus Pallas 1771.

Pedicellina echinata Hassell 1841; Goodsir 1845; Nitsche 1870.

P. hirsta Water 1918.

P. cernua Kirkpatrick 1888; Jullien et Calvet 1903; Osburn 1910.

Stolon slender. Calyx usually gibbous.



Text-fig. 2.
Pedicellina cernua (Pallas) ×25

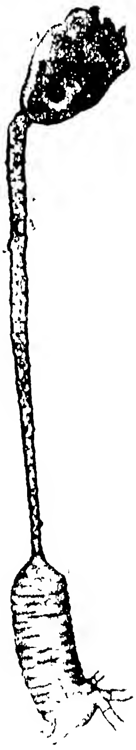
Tentacles about 15. Stalk stout, tapering gradually towards the upper end. A number of stout spines present on both of stalk and calyx. The specimens were secured together with that of Barentsia discreta growing on the test of a solitary Ascidian.

3. Barentsia discreta (Busk)
(Text-fig. 3)

Synonyms

Ascopodaria discreta Busk 1886
Barentsia discreta Kirkpatrick 1890; Waters 1905; Osburn 1910; Annandale 1912; Harmer 1915; Okada & Mawatari 1938.
Ascopodaria misakiensis Oka 1890.
Barentsia misakiensis Oka 1895.

Size large, reaching a total length of 4.5 mm. or more. Tentacles about 20. Stalk becoming slightly wider towards the upper end, and is covered with a thick cuticle. The inner layer of the stalk is penetrated by numerous minute funnel-shaped pores. Basal muscular portion of the stalk is white in colour. Stalk and stolon are light brown in colour. The cuticle of the stalk varies in colour and in thickness with the age of the specimens and with the localities where the specimens were taken. The present species has the most wide distribution among the members of this group. With the naked eye the present species can be easily distinguished from other members of Barentsia firstly, by the large size especially by the large basal muscular portion, looking always white in colour, and secondly, by the stalk which is long and narrow and of light brownish colour.



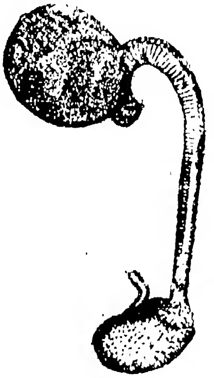
Text-fig. 3.
Barentsia discreta (Busk) ×12

4. Barentsia laxa Kirkpatrick
(Text-fig. 4)

Synonyms

Barentsia laxa Kirkpatrick 1890, Harmer 1915.

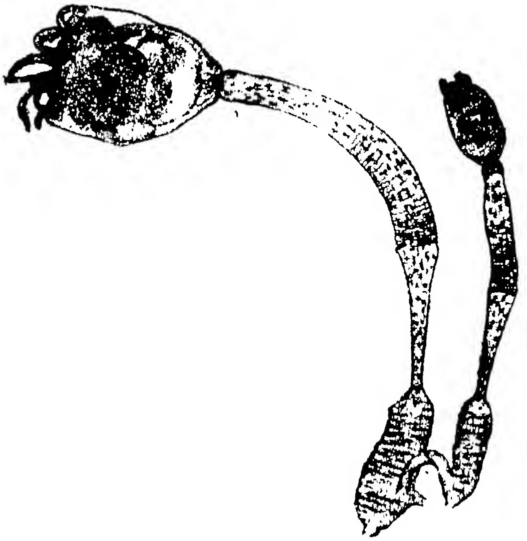
The stalk is narrow in basal part becoming broader towards the tip. The cuticle of the stalk becomes thinner towards the tip, is minutely annulated, and very flexible. The contractility is not confined to the basal muscular portion of the stalk, but is also present in the greater part of the length of the stalk. This species was found abundantly in Matsushima Bay in September and October growing on algae, oyster shell and on the test of solitary Ascidian. In another region of the Pacific coast of Miyagi Prefecture the writer obtained only one specimen forming a very small and young. It was secured from Shizugawa Bay.



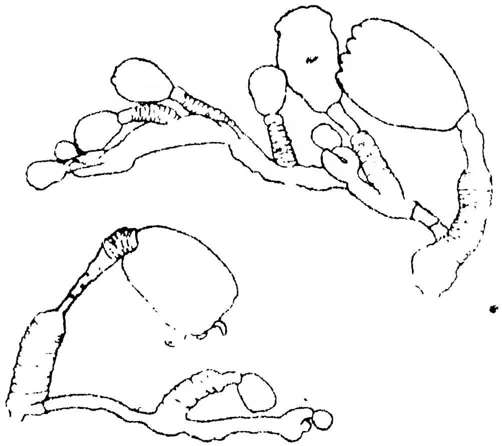
Text-fig. 4.
Barentsia laxa Kirkpatrick ×25
Young polypide. The colony, the material of the present report, is consisted of these young polypides.

5. Barentsia hozawai n. sp.
(Text-figs. 5, 6)

Size, large, attaining a total length of nearly 4 mm. Polypides arise from a jointed, creeping stolon. The expanded muscular portion at the base of the stalk is markely annulated. Stalk cylindrical, and the lower half is chitinous while the upper half is membranous. Chitinous portion of the stalk, light yellowish-brown in colour, is extremely slender below but expands considerably towards the tip, and is provided with many pores funnel-like in shape.



Text-fig. 5. Barentsia hozawai n. sp. ×25



Text-fig. 6. Barentsia hozawai ×25
young polypides and buds

They are seen to penetrate the inner layer of the stalk but not the outer, as is the case in B. discreta. The membranous portion of the stalk tapers gradually towards its upper end. The upper part is smooth but the lower part is minutely wrinkled. This membranous part of the stalk seems to be very flexible. Calyx large and slightly gibbous. Tentacles are 25-26 or more in number. Resting buds are unknown.

The characteristics of the stalk are markedly different from those seen in the previous species.

Professor Hôzawa collected this specimens from Izushima in Onagawa Bay. He has found the same growing on the test of a solitary Ascidian located in shallow water.

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SOCIOLOGICAL STUDIES OF THE PINE FORESTS IN JAPAN, ESPECIALLY WITH REGARD TO THEIR STRUCTURE AND DEVELOPMENT.[1]

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(Received December 20, 1948)

Extensive areas of Japan are occupied by pine forests which are composed of two-leaved pines, namely Pinus densiflora (Japanese red pine, "Akamatsu") and P. Thunbergii (Japanese black pine, "Kuromatsu"). The distribution of these pines is so wide that they can be found everywhere in our country, therefore various communities of pine trees can be distinguished according to their habitat conditions. The development of natural, semi-natural, or cultivated pine forests is also to be seen here and there.

Few studies about the facts mentioned above, however, have been done so far. Accordingly the author has carried out sociological studies of the pine forests, especially as to their structure and development while travelling over extensive areas in our country.

Distribution and Ecological Character of Pinus Densiflora

P. densiflora is distributed over an exceedingly wide area ranging from the southern part of Hokkaidô (42°40′N.) where it is very doubtfully indigenous, through Honshû, Shikoku and Kyûshû, to Yakushima Island (30°20′N.) in Kagoshima Prefecture. That is, it flourishes both in the zone of deciduous summer forests and in that of evergreen forests. It also grows everywhere in Korea and extends into eastern Manchuria (44°20′-45°20′N.) to the north and into Shantung Peninsula (36°N.) to the west. The altitudinal distribution is also very extensive, ranging from the coastal land to the mountain region. The upper limit of its distribution in Honshû is in the altitude of 500-1000 m. in the northeast and 1000-1600 m. in the southwest. That is, it can be found as far as the mountain region where Japanese beech, Fagus crenata, establishes itself as the climatic climax community. It may be easily understood from the facts mentioned above that P. densiflora is one of the most widespread trees in Japan.

Near the northern limit of the Asiatic continent, Botankô in southeastern Manchuria, the mean annual temperature is 2.6°C., the mean monthly temperature of January is—21°C., and that of July is 22.2°C. There are five months in which the mean monthly temperature

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[1] Contributions from the Mt. Hakkôda Botanical Laboratory. No. 32.

rises above 10°C. At Kagoshima in the Kyûshû district, close by the southern limit, the mean temperatures are 16.6°C. (annual), 6.7°C. (January), 26.9°C. (August), and nine months respectively. P. densiflora is not found on the Izu Islands where the mean monthly temperature of each month is always higher than 10°C. These facts show us that it is able to grow in a wide range of temperature.

P. densiflora occupies an extensive area in the drier parts of the country where the annual rainfall ranges from 1000 to 1500 mm., whereas it is limited in the moister areas with a precipitation of 1500-3000 mm. It grows even in the dry continental district of North China where the annual rainfall is about 600 mm., though it is not found in the driest districts. On the contrary it is rarely found in regions with a precipitation of more than 3000 mm.

P. densiflora can grow on various kinds of soil, viz. clay, loam and sand, and is found even on peat bogs and solfataras. Such indifference to the type of soil, as mentioned above, is due to its particular character requiring little mineral nutriment and soil moisture, and enduring higher soil acidity. Although it is able to grow on any soil, its best growth is attained on light rather than on heavy soil. These facts show us that the growth of P. densiflora is influenced more by the physical properties of the soil than by the chemical properties. The growth of pine forest on such impoverished stands as ridges, rocky out-crops, volcanic ejecta and alluvial sands, where other trees do not grow successfully, is due to its non-exacting characters.

Distribution and Ecological Characters of Pinus Thunbergii

P. Thunbergii is distributed throughout more restricted areas than the former species. Its northern limit in Japan is at the extremity of Shimokita Peninsula (41°30′N.) in the northern part of Honshû, and on the continent as far as the middle of the western coast of Korea (37°50′N.). It is found in the districts of Honshû, Shikoku and Kyûshû, growing more abundantly in the southern part, while less abundantly in the northern part. The southern limit is on Akusekishima Island which is situated near to Yakushima Island, the southern limit of P. densiflora. It grows luxuriantly on the Izu Islands, as far as Hachijojima Island (33°N.).

As to the altitudinal distribution, it is restricted to the coastal lands in the cooler regions, namely Tôhoku, Kantô and a part of Chûbu, though it extends to the mountain region about 800 m. above sea level in the warmer regions, namely Shikoku and Kyûshû. It can be seen accordingly that the most suitable area of growth of P. Thunbergii is in the warm temperate zone and extends therefrom into the cold temperate zone in a narrow belt along the coast.

At Aomori, near the northern limit of P. Thunbergii in Japan, the mean annual temperature is 9.2°C., the mean monthly temperature of January is -2.8°C., that of August is 22.9°C. and there are six months in which the mean monthly temperature rises over 10°C. At Jinsen, near the northern limit of P. Thunbergii in Korea, the mean temperatures are 10.7°C., -3.1°C., 24.9°C. and six months respectively. The temperatures at the southern limit of P. Thunbergii are presumed to be the same as those of P. densiflora because the southern limits of the two species are near to each other. It grows luxuriantly on the Izu Islands, where the mean monthly temperature of any month does not fall lower than 10°C. P. Thunbergii prefers a habitat with a greater amount of rainfall and higher air humidity to one with a smaller amount of rainfall and lower humidity. It is easy to understand from the above-mentioned facts that P. Thunbergii, unlike P. densiflora, is a tree inhabiting districts affected by oceanic climate.

This pine, like P. densiflora, can take growth on various types of soil. Its best growth, however, is attained in the lighter soils with richer mineral nutriment and higher exchangeable calcium. That is, it is more exacting than the other species.

Sociation Types and Associations of Our Pine Forests

The author studied the structures, developments and habitat conditions of our pine forests over an extensive area ranging from Aomori Pref., the northern end of Honshû, to Kagoshima Pref., the southern end of Japan. Numerous sociations are distinguished mainly by differences in the floristic compositions and they are, then, classified into sociation types according to their structures, physiognomies and habitat conditions. Several sociation types with similar structures and physiognomies are included in a larger unit, viz. association. Accordingly sociation type is a unit of a plant community between sociation and association, expressing ecological significance as well as structure.

In our pine forests, sociation types (S.T.) and associations are differentiated as follows:

A. Pinus densiflora—Quercus serrata Association

1. **Pinus densiflora-Quercus serrata-Rhododendron Kaempferi S.T.** The characteristic feature of this S.T. is the abundance of Rhododendron Kaempferi in the field layer. In undisturbed forests such deciduous trees as Quercus serrata, Q. crispula, Castanea crenata and Prunus serrulata var. spontanea are found with the pine. It occurs usually on loam and clay, but rarely on sand. The growth of the pine is usually well and it is sometimes excellent. The forests of this S.T. occupy extensive areas in the Tôhoku district and also in the inner parts of both of the Kantô and the Chûbu districts where the deciduous summer forests establish themselves as the climatic climax.

2. **Pinus densiflora-Quercus serrata-Pleioblastus chino S.T.** This S.T. has in the shrub layer a dense cover of Pleioblastus chino, Arundinaria ramosa and other similar dwarf bamboos of the same genera. Abundant growth of accompanying deciduous trees is found in the undisturbed forests as in the former S.T. It becomes established on the deep, sandy and clay loams of alluvial or diluvial origin. The growth of the pine is well. It is distributed chiefly on the Pacific side from the middle of Iwate Pref. southwards, through Miyagi and

Fukushima Prefs., to Tochigi and Ibaraki Prefs. This S.T. is found frequently in the deciduous forest zone and occasionally in the northern part of the evergreen forest zone.

3. Pinus densiflora-Quercus crispula-Sasamorpha spp. S.T. Quercus crispula, instead of Q. serrata, associates with the pine and such evergreen shrubs as Pieris japonica and Bobua myrtacea which are abundant in the shrub layer. The characteristic feature of this S.T. is that Sasamorpha spp. occupies the field layer. It is found occasionally in the mountain region of central and southern Japan.

4. Pinus densiflora-Quercus serrata-Rhus ambigua S.T. Quercus serrata, Prunus serrulata var. spontanea and sometimes Cornus controversa associate with the pine in the tree layer, and Palura japonica, Ilex crenata and Stephanandra incisa are abundant in the shrub layer. It is characteristic that Rhus ambigua dominates over the field layer. It occurs on deep, light soils such as volcanic ejecta and granitic soil. The pine trees of this type generally show so excellent growth that it is not too much to say that the greater part of the representative pine forests of P. densiflora in the cold temperate zone belong to this type. It is distributed widely in the Tôhoku district and sometimes in the mountain region of the Chûbu and the Chûgoku districts.

5. Pinus densiflora-Pinus pentaphylla S.T. A five-leaved pine, Pinus pentaphylla, commonly associates with P. densiflora. Ericaceous small shrubs, such as Menziesia cililcaryx var. multiflora, Hugeria japonica and Botryostege bracteata are abundant in the field layer. Herbaceous plants, however, are scarce. The pine forests of this S.T. exist as the edaphic climax community on the ridges at the lower part of mountains where the forests of Fagus crenata occupy extensive areas as the climatic climax. This S.T. is so rare that only two examples were found hitherto in the Tôhoku district.

6. Pinus densiflora-Quercus serrata-Eurya japonica var. montana-Rhododendron Kaempferi S.T. Deciduous trees are found accompanying the pine as in the forests mentioned above, and evergreen shrubs such as Eurya japonica var. montana, Camellia japonica var. spontanea and Pieris japonica are frequent. Rhododendron Kaempferi is dominant in the field layer, accompanied by evergreen Bladhia japonica and Skimia japonica. It is found on the loamy and clay soils, showing fairly good growth in the former and inferior growth in the latter. It is distributed in the transitional zone between the cold and the warm temperate climates of the Tôhoku district, namely in the coastal lands of Miyagi and Fukushima Prefs.

7. Pinus densiflora-Cryptomeria japonica S.T. Cryptomeria japonica is found commonly associating with the pine. In the field layer Sasa spp. is usually abundant, and Rhododendron Kaempferi and Hugeria japonica are frequent. Shizocodon uniflora occurs frequently in the field layer of this S.T. It is found on the deep, moist soils on the Japan Sea side from Akita to Shiga or Kyôto Pref. where snow falls heavily. The growth of pine trees in this S.T. is fairly good.

8. Pinus densiflora-Quercus serrata-Sasa spp. S.T. As accompanying trees, Quercus, Castanea and Prunus are abundant, and sometimes Betula platyphylla var. japonica is found. That the field layer is densely occupied by Sasa spp. is the most noticeable feature of this S.T. It is distinguished from the former S.T. by the absence of Cryptomeria, though the two S.T. are similar in respect to their structures. It develops on the various soils in the mountain region of the Tôhoku district.

9. Pinus densiflora-Quercus serrata-Hugeria japonica S.T. Q. serrata is abundant in company with the pine. The shrub layer is poor. It is characteristic that the small ericaceous shrubs dominated by Hugeria japonica are found in the field layer. This S.T. occurs on alluvial sands or mountain ridges where the soils are dry and poor. The growth of the pine is usually well, though it is inferior on the heavy clays. This S.T. becomes established frequently in the cold temperate zone, especially on the Japan Sea side.

10. Pinus densiflora-Juniperus rigida-Rhododendron Kaempferi S.T. No deciduous trees associating with the pine are found in the tree layer or subordinate tree layer, and it is characteristic that Juniperus rigida is abundant in the shrub and field layers. Small ericaceous shrubs, Rhododendron Kaempferi and Hugeria japonica, are also abundant. Herbs, on the contrary, are very rare. These forests are found on the shallow, poor, dry soils on ridges and other denuded places, and the growth of the pine is generally inferior. It is distributed in the drier parts of the cold temperate zone, viz. Miyagi, Fukushima, Yamanashi and Nagano Prefs.

11. Pinus densiflora-Pinus Thunbergii-Quercus serrata S.T. P. Thunbergii is found associating in abundance with P. densiflora, and deciduous trees such as Q.crispula, Q. serrata and Q. dentata are found in the subordinate tree layer. Rhododendron Kaempferi and Sasa spp. are frequent in the field layer. It occurs on loamy and clay soils along the coast of Aomori and Akita Prefs. on the Japan Sea side.

12. Pinus densiflora-Fraxinus Sieboldiana var. serrata S.T. No deciduous trees except Fraxinus are found in the tree layer. Such light requiring plants as Lespedeza Buergeri and Miscanthus sinensis are abundant in the field layer. It is seen on the shallow, poor, dry soils of ridges and other denuded places in the northern part of the Tôhoku district, chiefly in Iwate and Aomori Prefs. The growth of the pine is worse.

13. Pinus densiflora-Alnus pendulata S.T. The characteristic feature of this S.T. is that Alnus pendulata, an intolerant pioneer tree, is prevalent in the shrub layer. Quercus and Castanea are never found in company with the pine. The forests of this type are primary communities developing on the volcanic ejecta. The growth of the pine is fairly good on pumices, lapilli, ashes and mud flows, but extremely poor on lava flows. The author found the forests of this S.T. on Mt. Bantai in Fukushima Pref. and on Mt. Asama, in Nagano Pref.

14. Pinus densiflora-Deutzia crenata S.T. No deciduous trees are found in the tree layer in company with this pine, but numerous shrubs such as Deutzia crenata, Stephanandra incisa and Prunus incisa, etc., are luxuriant. This S.T. is found on the volcanic ejecta at the northern foot of Mt. Fuji, Yamanashi Pref. "Suwanomori", a pine forest famous for its excellent growth established itself on the mud flow. It develops also on the lava flows.

15. Pinus densiflora-Coriaria japonica S.T. This S.T. is devoid of any associating tree and shrub and shows a very simple structure. The field layer is also simple in structure, though Coriaria japonica, a typical non-exacting species, occurs in abundance. It is found on the infertile sands in both the coastal and the inland stands. The growth of the pine is well or slightly inferior. This S.T. is distributed here and there in the Tôhoku district.

16. Pinus densiflora-Salix spp. S.T. Willows such as Salix Gilgiana and Toisus Urbaniana are abundant as the subordinate trees, and Miscanthus sinensis, Pleioblastus chino and Arundinaria ramosa are frequently found in the field layer. It comes out as a primary forest on the dry, coarse, sandy deposits along rivers. It is distributed chiefly in the Tôhoku district and also in the inland districts of central Honshû.

17. Pinus densiflora-Sphagnum spp. S.T. This is an open community of stunted pines accompanied by marsh plants such as Alnus japonica var. arguta and Phragmitis longivalvis. The characteristic plant is Sphagnum spp. which covers the ground densely. It occurs on the bogs here and there in our country. The growth of the pine is so poor that it grows to a height of only 2 meters.

18. Pinus densiflora-Miscanthus sinensis Zoysia japonica S.T. The structure of this S.T. is simple, having few associated trees and shrubs. The field layer is occupied by such graminous plant as Miscanthus sinensis or Zoysia japonica, which is the characteristic feature of this S.T. The forests of this type are communities developing either on places denuded by grazing, or on exposed, bare lands. It is found everywhere in the temperate zone of our country, though on a small scale.

Pinus densiflora-Quercus serrata Association. All the S.T. mentioned above are found chiefly in the cold temperate zone, while a part of them are also found in the mountain region of the warm temperate zone. The principal accompanying trees are deciduous ones, indigenous to the cold temperate zone, such as Quercus serrata, Q. crispula, Castanea crenata and Prunus serrulata var. spontanea. Of these Q. serrata is the commonest and may be recognized as a characteristic species of the zone. The field layer is composed of such small ericaceous shrubs as Rhododendron Kaempferi and Hugeria japonica and such dwarf bamboos as Pleioblastus chino and Sasa spp. The pine forests of this zone, if they are left undisturbed, tend to become transformed into deciduous forests composed of the above-mentioned trees. All these sociation types, accordingly, may be included in the Pinus densiflora-Quercus serrata Association.

B. Pinus Thunbergii-Quercus crispula Association

Pinus Thunbergii-Quercus crispula S.T. In the subordinate tree layer of this S.T. there is an abundance of deciduous trees such as Q. crispula, Q. serrata, Q. dentata and Tilia japonica. Shrubs are rare. Grasses such as Miscanthus sinensis and Festuca rubra are abundant in the field layer. The forests of this S.T. exist exclusively on the sand dunes along the coast of the Japan Sea in Yamagata and Akita Prefs. The growth of P. Thunbergii is quite well.

Pinus Thunbergii-Quercus crispula Association. The characteristic feature of Pinus Thunbergii-Quercus crispula S.T. is an abundant growth of oaks, especially Q. crispula. Although no other S.T. similar to this one are to be found anywhere, the Pinus Thunbergii-Quercus crispula Association may be distinguished by its remarkable feature.

C. Pinus Thunbergii-Machilus Thunbergii Association

1. **Pinus Thunbergii-Machilus Thunbergii-Rapanaea neriifolia S.T.** Machilus Thunbergii and other evergreen trees, Cyclobalanopsis glauca and Cinnamomum Camphorea, are found in abundance associating with P. Thunbergii. The shrub layer is dominated by such evergreen shrubs as Rapanaea neriifolia, Eurya emerginata, Pittosporum Tobira, Vaccinium bracteatum, etc., of which the first two species are restricted to the area of this S.T. It occurs on the coastal dunes of the southwest, the warmest, most rainy district in Japan, as the most stabilized coastal forests of P. Thunbergii.

2. **Pinus Thunbergii-Machilus Thunbergii-Pittosporum Tobira S.T.** Machilus Thunbergii, Shiia spp. and Cinnamomum japonicum are found in abundance associating with P. Thunbergii. With the exception of Pittosporum, Eurya japonica var. montana, Ligustrum japonicum and Camellia japonica var. spontanea are abundant in the shrub layer, while Rapanaea neriifolia and Eurya emerginata are not seen. It becomes established extensively on the coastal land of the warm temperate zone.

3. **Pinus Thunbergii-Quercus phylliraeoides S.T.** This is an open community composed of stunted P. Thunbergii. It is characteristic that evergreen Quercus phylliraeoides is abundant in the shrub or subordinate tree layer. Rapanaea and Eurya are also found occasionally. It occurs chiefly on the shallow, dry soil of coastal cliffs in the districts of the southwest. It may be regarded as the edaphic climax community.

4. **Pinus Thunbergii-Neolitsea Sieboldii S.T.** Abies firma and Prunus serrulata var. spontanea are abundant in the tree layer, and such evergreens as Neolitsea Sieboldii, Machilus Thunbergii, Daphniphyllum macropodum, etc. constitute the subordinate tree layer. Eurya japonica var. montana and Camellia japonica var. spontanea are abundant in the shrub layer, while Pittosporum Tobira is rare. It is found on the coastal area of the northern half of Fukushima Pref.

5. **Pinus Thunbergii-Machilus Thunbergii-Quercus serrata-Eurya japonica var. spontanea S.T.** Such deciduous trees as Quercus serrata, Q. crispula and Zelkowa serrata, as well as Machilus, are abundant. Evergreen shrubs, however, as Eurya, Camellia, Pittosporum, etc. are exceedingly abundant in the shrub layer. It is distributed on the coastal area of the transitional regions between the warm temperate and the cold temperate zones, viz. Miyagi Pref., the southern part of Iwate Pref. and Noto Peninsula in Ishikawa Pref.

6. **Pinus Thunbergii-Machilus Thunbergii-Acer mono var. eupictum-Camellia japonica var. spontanea S.T.** Deciduous trees, Acer mono var. eupictum and Zelkowa serrata, are found in abundance associating with P. Thunbergii, while Machilus Thunbergii is occasional. Camellia japonica var. spontanea and

Pseudosasa japonica are abundant in the shrub layer, though Eurya and Pittosporum are rare. It occurs in the coastal land on the Japan Sea side in Yamagata and Akita Prefs.

Pinus Thunbergii-Machilus Thunbergii Association. The greater part of the stabilized, coastal pine forests of P. Thunbergii belongs to any of the above-mentioned seven sociation types. Although they differ from each other considerably in structure, their components express a common oceanic character. The constant occurrence of Machilus Thunbergii associating with P. Thunbergii through these sociation types is the most characteristic feature They may, accordingly, be united into the Pinus Thunbergii-Machilus Thunbergii Association. This association is found in the coastal lands of Kyûshû, Shikoku and the greater part of Honshû except the northern half of the Tôhoku district. That is, this association is the representative of the coastal forest of P. Thunbergii occupying extensive areas on the coast in Japan

Coastal Pinus Thunbergii Alliance. As all the stable communities of the Japanese coastal pine forests belong to any of the above-mentioned two associations of P. Thunbergii, viz. the Pinus Thunbergii-Quercus crispula Association and the Pinus Thunbergii-Machilus Thunbergii Association, we may call them together the Coastal Pinus Thunbergii Alliance. But it must be noted here that there are other sociation types which were not included in the Alliance because the forests of these sociation types were either young and developing into any of the association types or deteriorating from them. These unstable communities are as follows:

1. **Pinus Thunbergii-Imperata cylindrica var. Koenigii S.T.** The structure of this pine forest is very simple. Neither trees nor shrubs are found associating with P. Thunbergii, while Emperata cylindrica var. Koenigii and other graminous plants grow in the field layer. It is the pine forest that develops in front of sand beach and dune, and its distribution ranges from the northern part of the Tôhoku district to the southern part of the Kyûshû district.

2. **Pinus Thunbergii-Juniperus conferta S.T.** This S.T. is also of simple structure, like the former, and is occupied by a creeping juniper, Juniperus conferta, in the field layer. Its ecological character and distribution are similar to those of the former S.T. Forests of P. densiflora of almost the same structure as this S.T. are found on the sandy beaches of Niigata and Miyagi Prefs.

3. **Pinus Thunbergii-Vitex rotundifolia S.T.** The structure of the S.T. is also simple. Vitex rotundifolia creeps over the field layer instead of Juniperus. It is found developing in front of the sea shore and is distributed from Aomori to Kagoshima Pref.

4. **Pinus Thunbergii-Hypnum spp. S.T.** The structure of this S.T. shows a simpler structure than any other mentioned above. In the field layer a few graminous plants grow, while in the ground layer Hypnum spp. are frequently found, which is the characteristic feature of this S.T. It is a degraded S.T. owing to the influence of man upon the vegetation, and is found on the moist, stabilized dunes in the greater part of our coastal area.

D. Pinus densiflora-Cyclobalanopsis glauca Association

1. Pinus densiflora-Cyclobalanopsis glauca-Dicranopteris dichotomata S.T. The typical forest of this S.T. has a subordinate tree layer composed of such evergreen broad-leaved trees as Cyclobalanopsis glauca, Shiia Sieboldii, Bobua japonica, etc. The shrub layer consists of various, evergreen shrubs, and the field layer is occupied by evergreen ferns such as Dicranopteris dichotomata and D. glauca. Sometimes the associating trees and shrubs are removed by frequent cutting, and only the field layer occupied by Dicranopteris is left. It flourishes extensively on various soils through the warm-temperate zone, and may be regarded as the representative pine forest of this zone.

2. Pinus densiflora-Cyclobalanopsis glauca-Eurya japonica var. montana S.T. As associating trees Cyclobalanopsis glauca and Shiia Sieboldii are abundant and Quercus serrata grows frequently in the northern districts. Evergreen shrubs, Eurya japonica var. montana, Ilex pedunculosa, Vaccinium bracteatum, Pieris japonica and Rhododendron reticulatum, occur in the shrub layer. The field layer is poor. It establishes itself on ridges and steep slopes in the northern part or in the mountain region of the warm temperate zone. Although the growth of pine is usually not quite well, a few excellent cases can be found at Ôdô, in Kôchi Pref. and Tokikuni in Ishikawa Pref.

3. Pinus densiflora-Cyclobalanopsis glauca-Pleioblastus yoshidake S.T. Cyclobalanopsis glauca and Ilex pedunculosa are found in abundance mixing with the pine in the tree layer. Pleioblastus yoshidake occupies the field layer, which is the characteristic feature of this S.T. It occurs on deep moist soils, showing excellent growth on light, sandy loam. It is found frequently on the plains and hills in the warm temperate zone, and it shifts gradually into P. densiflora-Q. serrata-Pl. chino S.T. of the cold temperate zone.

4. Pinus densiflora-Tsuga Sieboldii S.T. Tsuga Sieboldii and other conifers such as Abies firma and Chamaecyparis obtusa associate with the pine, though evergreen broad-leaved trees are rare. The shrub layer is densely occupied by evergreen shrubs, while the field layer is very poor. It is seen on ridges and steep slopes in the warm temperate zone. This type may be regarded as the edaphic climax community.

5. Pinus densiflora-Eurya japonica var. montana-Hugeria japonica S.T. Although the physiognomy and structure of this S.T. are similar to those of Pinus densiflora-Cyclobalanopsis glauca-Eurya japonica var. montana S.T., it is distinguished from the latter by the abundance of ericaceous shrubs as Hugeria japonica and Vaccinium hirtum in the field layer. It is found on the poor, acid soils of alluvial sands or of mountain ridges. Accordingly the growth of the pine is usually worse. It is distributed exclusively in the neighbourhood of Kyôto in central Japan.

6. Pinus densiflora-Cyclobalanopsis acuta-Eurya japonica var. montana S.T. An evergreen oak, Cyclobalanopsis acuta, is abundant in the tree layer and such deciduous trees as Acer and Carpinus also occur. Camellia japonica var. spontanea is abundant in the shrub layer and Bladhia japonica in the field layer. It is found in the lower mountain region of southwestern Japan.

7. Pinus densiflora-Pinus Thunbergii-Juniperus rigida-Eurya japonica var. montana S.T. This is a community of stunted pines which grow to the height of only 3 m. even in the course of 100 years. P. Thunbergii is found in abundance with P. densiflora. Juniperus rigida, Eurya japonica var. montana, Rhododendron dilatatum, Rh. linearifolium var. macrosephalum, Pleioblastus yoshidake and Miscanthus sinensis are found frequently as undergrowth, of which Juniperus is the most characteristic. It establishes itself on shallow, poor, dry soils chiefly in the districts around the Seto Island Sea where the climate is dry for Japan. This is the typical, inferior pine forest of the warm temperate zone just as Pinus densiflora-Juniperus rigida-Rhododendron Kaempferi S.T. is the typical of the cold temperate zone.

8. Pinus densiflora - Rhododendron kiusianum-Miscanthus sinensis S.T. This is a younger, developing pine forest and has no accompanying trees and shrubs. In the field layer Rhododendron kiusianum and Miscanthus sinensis are abundant. It is found developing on the ranges and burned places in the Kyûshû district.

Pinus densiflora - Cyclobalanopsis glauca Association. The above-mentioned eight S.T. are found generally in the warm temperate zone where evergreen broad-leaved forests establish themselves as the climatic climax. Evergreens are abundant in these S.T. That is, Cyclobalanopsis, Shiia and Ilex are abundant in the tree or subordinate tree layer, and Eurya, Vaccinium, Bobua and Camellia are abundant in the shrub layer. The field layer is also occupied by evergreen ferns like Dicranopteris. Considering the structures stated above, this group of S.T. may be safely called the Pinus densiflora-Cyclobalanopsis glauca Association. The components of the Association are mostly the same as those of the climatic evergreen broad-leaved forests which are chiefly composed of Cyclobalanopsis and Shiia.

E. Pinus Thunbergii-Cyclobalanopsis glauca Association

1. Pinus Thunbergii-Eurya japonica var. montana-Dicranopteris glauca S.T. Pinus Thunbergii is pre-dominant in the tree layer accompanied by P. densiflora and also by various evergreen trees such as Shiia japonica, Cinnamonum Camphorea, Machilus Thunbergii and Distylium racemosum. In the shrub layer Eurya japonica var. montana is abundant, and in the field layer Dicranopteris glauca and D. dichotomata grow in abundance. It occurs on deep, well-drained soils, chiefly in the Kyûshû district.

2. Pinus Thunbergii-Cyclobalanopsis glauca-Pleioblastus yoshidake S.T. Pinus densiflora, Cyclobalanopsis glauca, Machilus Thunbergii, Cinnamonum japonicum and Quercus variabilis are found frequently associating with P. Thunbergii. Eurya japonica var. montana are frequent in the shrub layer, and Pleioblastus yoshidake dominates the field layer. It establishes itself on deep alluvial or diluvial soils and also on volcanic ashes. It is distributed in the districts of Kyûshû, Shikoku and Chûgoku.

3. Pinus Thunbergii-Alnus Sieboldiana S.T. Alnus Sieboldiana is abundant in the shrub layer of this forest, and lithophytic, evergreen ferns as Nephrolepis cordifolia and Pyrosia lingua are frequent in the field layer. It is open pine forest developing on the lava and pumice ejected recently on Miyakejima Island.

4. Pinus Thunbergii-Deutzia Siebodii S.T. Few trees other than Pinus Thunbergii are found in the tree layer. Deutzia Sieboldii is abundant and Eurya japonica var. montana is frequent in the shrub layer, in which the young trees of Cinnamomum Camphorea, C. japonicum and Ilex Oldhami also occur. Daphniphyllum glaucescens, Nephrolepis cordifolia and Oplismenus patens grow vigorously in the field layer. It is found on the lava and pumice on Sakurajima Island in Kagoshima Pref., where the pine is, however, increasing by plantation.

Pinus Thunbergii-Cyclobalanopsis glauca Association. The four sociation types mentioned above are found in the southwestern parts of Japan, especially in the Kyûshû district. The tree layers of these pine forests are for the most part composed of P. Thunbergii, accompanied by P. densiflora. Many evergreen broad-leaved trees, Cyclobalanopsis, Shiia, Cinnamomum, Distylium and Machilus, are usually abundant in the tree or subordinate tree layer. Evergreen shrubs such as Camellia and Bobua are found in abundance in the shrub layer. Dicranopteris glauca and D. dichotomata spread over the field layer. Although they resemble those of the Pinus densiflora-Cyclobalanopsis glauca Association they are distinguished from the latter by the conspicuous abundance of P. Thunbergii and subtropic evergreens as Cinnamomum and Distylium. They may be, accordingly, called the Pinus Thunbergii-Cyclobalanopsis glauca Association. The forests of the latter two S.T. are in the course of development on the volcanic ejecta.

The Development of Japanese Pine Forests

It may be proved from the paleobotanical and the pollen analytical studies up to this time that both species of pines appeared in recent geological times. Their first occurrence was probably in the upper Pleistocene period. They were, however, rare at first and increased gradually. Nowadays the pines flourish extensively all over the country, which is chiefly due to the interference of man destroying the climatic deciduous or evergreen broad-leaved forests. That is, the expansion of the pine forests has been done at the sacrifice of the broad-leaved forests. The development of the pine forests in general is accomplished semi-naturally as mentioned above, though they also develop naturally or by plantation. The author investigated the various cases of development of our pine forests, travelling all over the land, with the following result:

A. Natural Development

As it is humid in our country, the natural development of pine forests are restricted to the stands of special edaphic conditions, viz. ridges, rocky outcrops, screes, volcanic ejecta and alluvial sands. These forests may be regarded as edaphic climax of pioneer communities. The following examples were seen:

1. Ridges and rocky outcrops. Although exacting trees can not establish themselves on such habitats, as the soils are shallow, dry and poor there, the pines are able to grow there vigorously and to form the forests which may be considered the edaphic climax community. Pinus densiflora-Pinus pentaphylla S.T. on the coast of Lake Towada, Aomori Pref., P. densiflora-Quercus serrata-Hugeria japonica S.T. on Kinkasan Island in Miyagi Pref., and P. densiflora-Tsuga Sieboldii S.T. in the southwestern part are examples of naturally developed forests on ridges or rocky outcrops.

2. Volcanic ejecta. As there are many volcanoes in Japan, we can find in our country places that have been newly laid out by volcanic ejecta, viz lava, pumice, ash and mud. Exacting trees are not able to establish themselves on such infertile soils, while the pines and other non-exacting trees can invade and establish themselves. The pioneer forests developed in these areas are occasionally composed of the pines, because they can grow more rapidly than any other trees. For example, in the cold temperate zone: Pinus densiflora-Alnus pendulata S.T. on the mud-flows on Mt. Bantai in Fukushima Pref., P. densiflora-Deutzia crenata S.T. on the lava- and mud-flows on Mt. Fuji in Yamanashi Pref., P. densiflora-Quercus serrata-Rhus ambigua S.T. on the pumice fields on Mt. Asama in Nagano Pref.; in the warm-temperate zone: Pinus Thunbergii-Alnus Sieboldiana S.T. on the lavas and pumices on Miyake-jima Island.

3. Alluvial sands and gravels. The pines usually prevail on alluvial sands and gravels, where other exacting trees are not able to establish themselves owing to the infertility and dryness of the site. P. densiflora-Quercus serrata-Hugeria japonica S.T. and P. densiflora-Q. serrata-Pleioblastus chino S.T. are found on such areas in the Tôhoku and and neighbouring districts. P. densiflora-Salix spp. S.T. is a community developing in newly deposited areas by the side of rivers. Almost all the pine forest which grow nowadays in the coastal sands were for the greater part originally planted, therefore they shall be mentioned in the section concerning artificial development.

4. Screes or collapsed areas. We can occasionally find pine forests developed on the bare areas which were caused by land collapses. The natural development of the pine forests accomplished there may be regarded as the commonest in our country. These forests, however, are small in scale. The S.T. developed on such areas are as follows: In the cold temperate zone: P. densiflora-Miscanthus sinensis Zoysia japonica S.T.; in the warm temperate zone: P. Thunbergii-Eurya japonica var. montana-Dicranopteris dichotomata S.T. as well as the former.

5. Bogs and solfataras. Groups of pines are found developing even on such extreme habitats as bogs and solfataras where other trees are not able to establish themselves owing to stagnation, high acidity and infertility of soil.

B. Semi-natural Development

Formerly both the evergreen and the deciduous broad-leaved forests had flourished over the greater part of our country. They were, however, destroyed by human agencies such as clearing, firing, grazing, etc. The pines are able successfully to invade and to reproduce themselves on these denuded places. Considerable areas which were at one time probably occupied by the broad-leaved forests came to be covered with regenerated forests of pine. The examples of this semi-natural development are as follows:

1. **Cleared areas.** Both the dediduous and the evergreen broad-leaved forests were, most probably, made extinct by repeated clearing in our country. Then, the pines were able to establish themselves in these denuded places. They tended gradually to form pure forests when other trees which hindered their development were removed. They could reproduce themselves more easily in areas where coniferous forests, especially pine forests formerly existed, than in areas of broad-leaved forests. The greater part of our pine forests were established in this way. The following S.T. are found developed in these cleared areas. In the cold temperate zone: P. densiflora-Quercus serrata-Rhododendron Kaempferi S.T., P. densiflora-Q. serrata-Pleioblastus chino S.T. and P. densiflora-Cryptomeria japonica S.T.; in the warm temperate zone: P. densiflora-Cyclobalanopsis glauca-Dicranopteris dichotomata S.T. and P. densiflora-Cy. glauca-Pleioblastus yoshidake S.T.

2. **Burned areas.** Relatively few cases in which the pine forests developed on burned areas are known in our country, while they are very frequent in other countries. In former days when forest fires were purposely started, the pine forests were developed frequently in this way. For example, P. densiflora-Cyclobalanopsis glauca-Eurya japonica var. montana S.T. at Odô in Kôchi Pref. is said to have been developed on such a burned area.

3. **Denuded grasslands.** Our grasslands which are used for grazing and mowing are made semi-naturally by clearing and firing the forests. If these grasslands are devastated by excessive use, the invasion and establishment of pines become very easy. These invading pines will develop gradually into a pine forest, if they are left to themselves. Many examples of this development can be seen in our country, and a number of excellent pine forests developed in this way, especially in the Tôhoku district. In the cold temperate zone the initial stage is P. densiflora-Miscanthus sinensis Zoysia japonica S.T. and the final one is usually P. densiflora-Quercus serrata-Rhus anbigua S.T.; in the warm temperate zone the initial stage is the same as in the former zone, the final stage, however, is P. densiflora-Cyclobalanopsis glauca-Pleioblastus yoshidake S.T.

4. **Abandoned fields.** We can occasionally find pine forests developed or developing on abandoned fields. The initial stage is usually P. densiflora-Miscanthus sinensis Zoysia japonica S.T. But the final stages vary according to the climate in which they established themselves. In the cold temperate zone: P. densiflora-Quercus serrata-Rhododendron Kaempferi S.T.; in the warm temperate zone: P. densiflora-Cyclobalanopsis glauca-Pleioblastus yoshidake S.T. and P. Thunbergii-Cy. glauca-Dicranopteris dichotomata S.T.

5. **Unsuccessfully planted areas.** Clearing off the vegetative cover and preparation of soil are usually done before the plantation. Bared patches, therefore, make their appearance here and there in these planted sites. The

pine trees are able to invade these areas easily, and develop themselves into pure, pine forests, especially in dry, impoverous habitats where the exacting planted trees can not establish themselves. These examples are found for the most part on the ridges of planted land everywhere in our country. They are seen usually on the planted sites of Chamaecyparis obtusa, Cryptomeria japonica, Cinnamomum japonica, etc.

6. Other bare areas. The pine trees often invade areas laid by man, viz. cleared and reclaimed lands, and they come gradually to be united into a pioneer community, P. densiflora-Miscanthus sinensis Zoysia japonica S.T.

C. Artificial Development (Plantation)

The artificial development of the pine forest is achieved by the plantations, which have been done considerably over wide areas in our country. Planting of the pines is done usually on waste lands like denuded ranges and coastal dunes. These planted pines not only establish themselves as a pine forest but are also naturalized in propagating in the neighbourhood. Two cases are known as particular examples of plantation on bad lands. In the districts around the Seto Island Sea an extensive area has been denuded by over-lumbering. P. densiflora and P. Thunbergii are replanted in this bare area, and in this case their establishment is attained successfully when Alnus pendula or Myrica rubra are planted with them. Nowadays almost all coastal dunes have been covered by pine forests, except in Hokkaidô, though they were once bare and attacked by sand drifts. P. Thunbergii were planted to keep the areas from the prevailing wind and sand drift, which was achieved with great effort. The greater part of these forests are naturalized and are reproducing themselves successively with the help of proper practices being used at present.

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A NEW EPIZOIC ALGA, CHLAMYDOMONAS HYDRAE, N. SP., FOUND ON THE FRESH-WATER POLYP.

BY

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(With 2 Text-figures)
(Received September 30, 1948)

The writer has previously reported (1947) on Chlamydomonas sp., a green alga, found epizoic on the fresh-water polyp. This green alga which was first discovered from a pond in Kyûshû has subsequently been found from several reservoirs in Miyagi Pref. and was collected abundantly from three lakes in Nagano Pref. They were found attached to three different species of the fresh-water polyp. This epizoic alga is a species new to science, and is named Chlamydomonas hydrae from its host.

The writer is indebted to the Late Prof. Sanji Hôzawa and to Profs. Mutsuo Katô and Arika Kimura for their kind valuable advices.

Chlamydomonas hydrae, n. sp.

The size of the cell in living specimens which were collected from three localities is as shown in Table 1 and Text-fig. 1.

The cell is elongate oval in form, narrowed towards the anterior end and more or less curved. The size and form of the cell somewhat resembles a large glutinant, a kind of nematocysts of the polyp.

The cell-wall is rather thin; and a very small papilla is present at the anterior end. The two flagella of equal length, almost as long as the length of

Table I. Size of the cell in living specimens from three localities.

Locality	Minimum		95% fiducial range of mean		Maximum	
	Length	Width	Length (μ)	Width (μ)	Length	Width
(1) Reservoirs in Miyato-shima (June, 1944)	11.1	3.9	12.24-12.94	3.90-4.16	13.7	4.6
(2) Reservoirs near Sendai (June and July, 1945)	9.8	3.3	11.81-12.43	4.00-4.36	13.7	5.2
(3) Lakes in Nagano Pref. (Oct., 1946)	11.7	2.6	12.95-14.13	3.83-4.21	16.9	5.2
Total	9.8	2.6	12.48-13.08	3.99-4.21	16.9	5.2

the cell, arise anteriorily from the side of the papilla. The two contractile vacuoles are situated in the anterior colourless cytoplasm. A small eye-spot is located in the anterior side of chloroplast. The cell possesses a concave lateral chloroplast, confined to its convex side. A marked pyrenoid is present in the middle or rather more anterior part of the cell (Text-fig. 2, A).

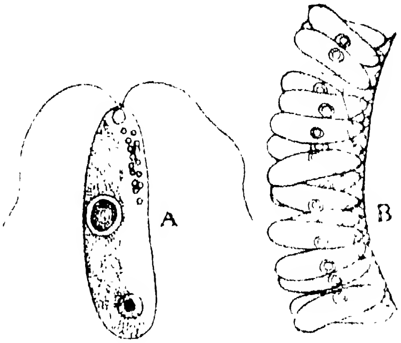
This alga seems to be normally epizoic. The organism attaches itself almost perpendicular to the body-surface of the polyp by the flagella which are curved backwards at the points of attachment (Text-fig. 2, B). The green alga infrequently transfers its position on the body-surface of the polyp, while it usually oscilates the body by means of the motion of its flagella. It was observed that the epizoites rarely become detached from their host in culture-media, even by strong shaking of the tentacles of the polyp, but on the slide glass, some epizoites sometimes detach from their host and mostly swim away making active spiral movements by rolling themselves. The algae attach over the whole body-surface of the polyp, and are commonly dense on the distal portion of the column and tentacles. When they attach sporadically, the algae are not isolated one by one, but assemble rather into groups.

L\W	1	2	3
1	\	○	●
2	○	\	●
3	○	○	\

Text-fig. 1. Comparison between the means of length (L) and width (W) of the cell of the specimens from three localities mentioned in Table 1 (in 95% reliability).

○ No significant difference between means.

● Significant difference between variances.



Text-fig. 2. Chlamydomonas hydrae, n. sp.

A, General form of the cell.

B, Cells attached to the body-surface of the fresh-water polyp.

A, ×2500; B, ×1000.

The green alga was found epizoic on the three species of fresh-water polyp, Hydra japonica, H. paludicola, and H. magnipapillata, and so it seems that the epizoite does not choose any particular species of the polyp. The physiological relation between the green algae and the polyp is not yet known. The host seems to suffer no visible influence by the thinly attached epizoites, but when their attachment is dense the body of the host may be somewhat smaller than the normal one in size. Unfortunately the life history of this alga has not been observed.

Localities and seasons of collection: A pond near Miyazaki, Miyazaki Pref. (June, 1943); a reservoir near Nakatsu, Oita Pref. (June, 1943); several reservoirs in Miyato-shima, Matsushima Bay, Miyagi Pref. (June, 1944); a pond near Hachirô Lagoon, Akita Pref. (Nov.

1944); two reservoirs near Sendai, Miyagi Pref. (June and July, 1945); Lake Kisaki, L. Nakatsuna, and L. Aoki, Nagano Pref. (Oct. 1946).

Remarks: The present new species closely resembles Chlamydomonas rattuli Korshikov found on a rotifer, especially in the epizoic mode of life. But the present Chlamydomonad can be distinguished from rattuli in the form of the cell, the presence of the papilla, and the position of arising of the flagella.

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THE MT. HAKKODA BOTANICAL LABORATORY.

BY
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(With 1 Plate)

(Received December 20, 1948)

The Mt. Hakkoda Botanical Laboratory was established in 1929 as a branch of the Biological Institute, Tohoku University in Sendai. The laboratory is situated at about 900 meter elevation on the southwestern slope of Odake, the highest peak (1,585 m) of Mt. Hakkoda. Mt. Hakkoda in reality is an extinct volcano group, consisting of eight main peaks, and stands on the northeastern end of Honshu, the main island of Japan, being located at 140 53 E.L. and 40 39 N.L.

Now, the laboratory buildings are situated in the amidst of the alpine botanical garden of some 10 hectare containing many native trees, shrubs and herbaceous perennials, besides numerous kinds of alpine plants collected from several high mountains and the north parts of this country. They are all well kept in the natural conditions and provided for the botanical study of the investigators as well as for the appreciation of the alpine plants lovers. Although the botanical garden is surrounded with a wide and luxuriant mixed vegetation of Sasa and shrubs, great many different types of native vegetation in this mountain region offers exceptional facilities for the study of plant ecology.

The very nature of the extinct volcano, weathering, erosion and other physiographical factors as well as remarkable diversity of habitat at every local place bring about the many different kinds of vegetation in a rather limited area: While the summits of the peaks are covered with a large number of alpine plants, their gentle slopes are occupied by coniferous and deciduous forests having Sasa-shrub as undergrowth. Solfataras and hot springs are still found in several places with their special vegetations. Bare region, rocky and detritus, of volcanic origin together with numerous streams originated from mountain snow which remains till the late summer, presents quite different habitats for the mountain plants. Still more conspicuous type of vegetation is that which is developed on the peat bogs and marshes on the terraces of the volcano. In short, Mt. Hakkoda offers great many types of native vegetation of which some are still remained untouched by the botanists. Thus in Mt. Hakkoda not only many different ecological types of vegetation are found within a day's walk from the botanical laboratory, but also there are a large number of interesting places illustrating a wide range of development of vegetation.

Indeed, the work of the Mt. Hakkoda Botanical Laboratory includes both botany and zoology, special attention being given to ecology and field works. The ecological work and exercise were first done in 1929 in this laboratory and has been carried on each summer since that time. The scope and nature of this foundation works are indicated by the list of publication cited below.

Any investigator or collector may be granted the use of the laboratory, under certain conditions, during summer time from the end of May to the beginning of October. Inquiries for this privilege and for information regarding the laboratory should be sent to the director of the laboratory Prof. Y. Yoshii in the Biological Institute, Tohoku University, Sendai, Japan.

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EXPLANATION OF THE PLATE

A. Botanical laboratory and a part of the garden.

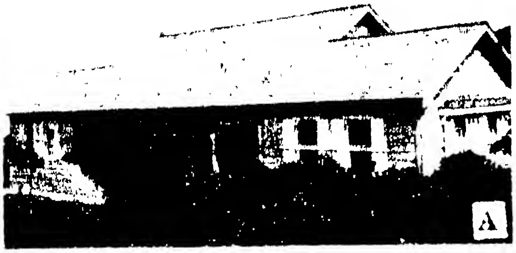
B. General view of the botanical garden in early summer, showing a laboratory building (behind) and a dormitory (in front).

C. Rock garden, Peak Isikura in the distance.

D. View of a swamp in the garden.

E. Peak Odake, viewed from the garden in autumn.

F. Natural bog in the garden (an ancient crater) Eriophorum vaginatum in the foreground.



A

B

C

D

E

F

Y. YOSHII: Mt. Hakkôda Botanical Laboratory.

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STUDIES OF CLEAVAGE. V. THE ROLE OF VACUOLES IN THE CLEAVAGE PLANE FORMATION IN SEA URCHINS' EGGS.*)**)

BY

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(With 20 Text figures)

(Received August 31, 1949)

Cleavage, division of cytosome, is of two types; namely by furrowing or constriction, and by the formation of a cell plate. Broadly speaking, the first is characteristic of mitosis in higher animals, particularly in the cleavage of the animal ovum, and the second of higher plants. Notwithstanding numerous reports on the cleavage of animal eggs, our knowledge on the mode of formation of the increasing surface of animal cells is very limited. In the previous paper it was stated that the cell surface of the cleavage plane in the sea urchins' eggs is a newly formed surface In the present paper it is intended to give the results of further observations on the mode of formation of cleavage plane in the sea urchins' eggs.

Observations were carried out on the eggs of *Temnopleurus harduickii* (GRAY), *Strongylocentrotus nudus* (A. AGASSIZ) and *Strongylocentrotus pulcherrimus* (A. AGASSIZ) at the Asamushi Marine Biological Station.

BEHAVIOR OF VACUOLES WITH BASOPHILIC GRANULES IN THE EGGS OF *TEMNOPLEURUS*.

The fertilized eggs of *Temnopleurus hardwickii* were fixed with BOUIN's fluid

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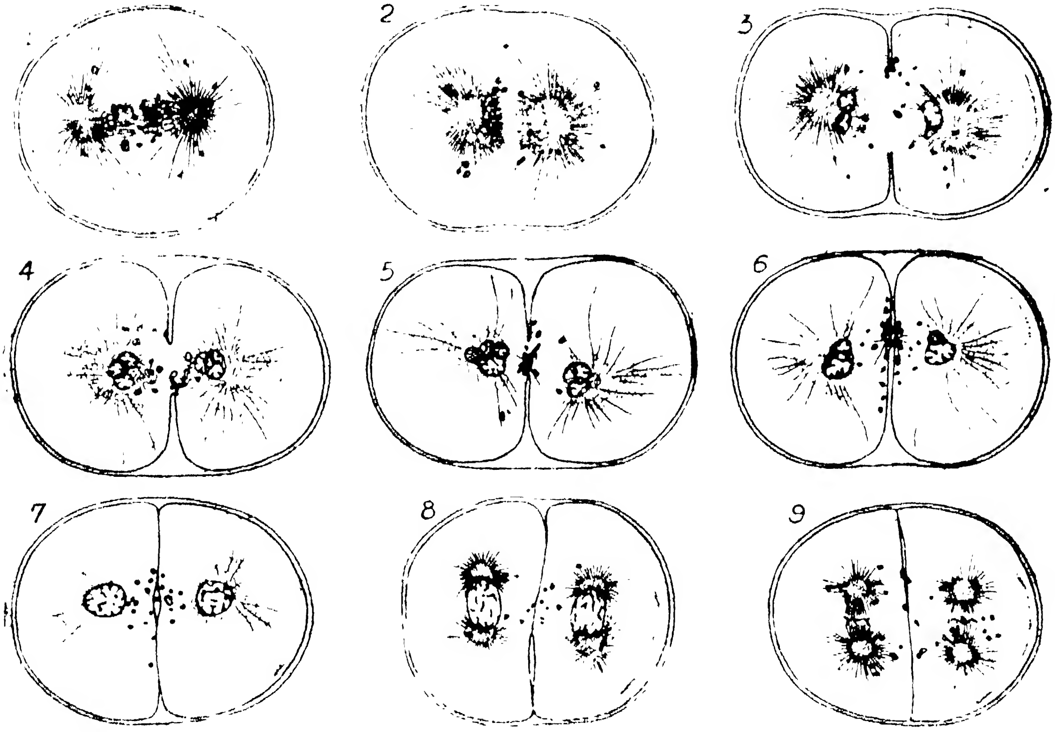
**) Contributions from the Marine Biological Station, Asamushi, Aomori Ken. No. 178.

at the first cleavage stage. The paraffin sections were stained by HEIDENHAIN's iron haematoxylin or by aqueous solution of Janus green after being washed for ten minutes with N/2 NaOH. The basophilic granules were electively stained with the latter method.

In the egg of *Temnopleurus* the first cleavage furrow begins to appear 40 minutes after fertilization. The interval between the first and second cleavage, which will be called the cleavage interval, is 20 minutes at 25°C. And it was noted from the observation of the movement of the surface of the egg during cleavage that the process of cleavage can be divided into three succeeding stages. The first one third of cleavege interval is the stage of surface extension in which the furrow is formed by extension of the cortical cytoplasm of the egg. In the middle one third the new cell surface is formed at the furrow region. The last one third is the karyokinetic stage of the next cleavage (MOTOMURA 1940).

The morphological changes during those three stages of the cleavage interval were studied on the stained sections At the anaphase of the nuclear division of the first cleavage, many vacuoles, which are empty at the center and are adhered with basophilic granules, were observed around the amphiaster. Those vacuoles will be called hereafter the basophilic vacuoles. At the telophase, when the division of the cytosome begins the basophilic vacuoles were coming to the spindle region. The cytoplasm of the equatorial region was porous at this stage, and formed the diastema, a more lightly stained zone composed of larger alveoli (Figs. 1 and 2). When the cleavage began, the basophilic vacuoles were observed mainly at the furrow tip, and some of them were observed near the spindle, which had begun to fade. Those phenomena were observed during the stage of surface extension (Fig. 3). At the end of this stage the basophilic vacuoles were moved to the equatorial region of the egg (Fig. 4).

The middle one third of the cleavage interval is the stage of new formation of the cell surface. And it was described in the previous paper that the surface area of the furrow region of the cell increased remarkably. At this time the basophilic vacuoles fused to each other, and formed large vacuoles at the equatorial region, especially at the middle of the spindle (Figs. 5 and 6). In some preparations it was also observed that the basophilic vacuoles were attaching to the cell surface at the cleavage plane, where the new formation of the cell surface was assumed, and the cell surface itself became also basophilic. At the last one third of the cleavage interval, the basophilic vacuoles mostly disappeared from the cleavage plane. And, a lens-shaped cavity, which is the first rudiment of the segmentation cavity, was observed between the two blastomeres (Figs. 7, 8 and 9). It will be assumed that the cavity will be formed by the released substance, either by the fluid of the vacuoles or by the osmotically active substance from the vacuole. At the anaphase of the nuclear division of the second cleavage the basophilic vacuoles appeared again around the cytaster.



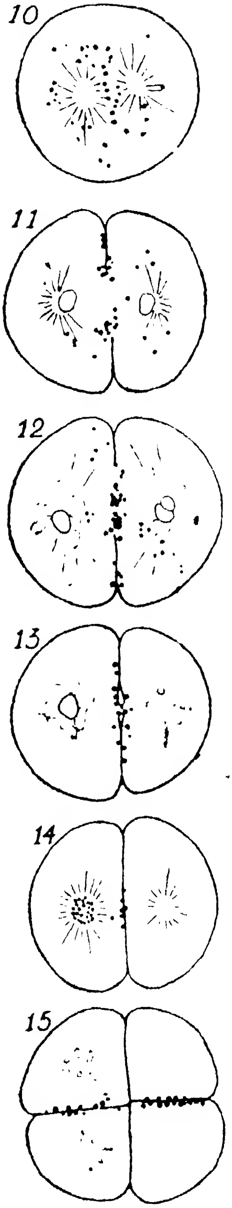
Figs 1—9. Sections of eggs of *Temnopleurus* stained by HEIDENHAIN's hematoxylin, showing the behavior of vacuoles during the course of the first cleavage. Fig 1 Anaphase of the nuclear division. The basophilic vacuoles are visible near the equator and cytasters. Fig. 2. Telophase. Fig. 3. Beginning of the furrowing, showing the basophilic vacuoles at the furrow tip and at the spindle region. Fig 4 Furrowing stage showing the vacuoles moved to the equatorial region of the spindle. Fig. 5 and 6. End of the constriction of the furrow, showing the vacuoles adhering to the cell surface of the cleavage plane. Fig. 7. End of telophase. The new formation of cell surface was mostly accomplished. A lens like space and a cell bridge are visible between the blastomeres Figs. 8 and 9. Beginning of the karyokinesis of the second cleavage. The lens-like space is present.

The behavior of the basophilic vacuoles reveals in the first place that they migrate to the equatorial region prior to the beginning of the formation of the new cell surface, and disappeared at the time when this phenomenon is accomplished. And, thus, they will be the material of the new cell surface. In the second place, it is also noticeable that at the end of the first cleavage a lens-like cavity, which is the beginning of the segmentation cavity is formed.

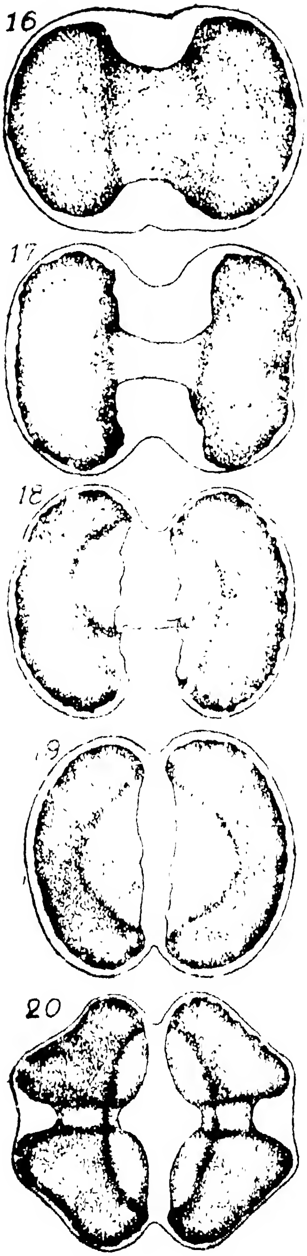
BEHAVIOR OF SILVER-STAINED GRANULES IN THE EGG OF *STRONGYLOCENTROTUS PULCHERRIMUS*.

In the egg of *Strongylocentrotus pulcherrimus* the silver-staining method after DAFANO was employed for the staining of the vacuoles, because they were not stained with the above mentioned method. The eggs were fixed by cobalt formol mixture after DAFANO at the cleavage stage. They were then impregnated with 1% silver nitrate, and were blackened with the hydroquinone and sodium sulphite developer. In paraffin sections the vacuoles were stained black. Whether or not those figures are to be comparable with the GOLGI apparatus, the writer is not certain because other methods for GOLGI apparatus were not tried. Nevertheless, in the present material this method was useful for demonstrating the special vacuoles of the egg of this species.

The behavior of the silver-stained vacuoles simulated that of the basophilic vacuoles in *Temnopleurus*. Shortly prior to the beginning of the furrowing, the silver-stained vacuoles were observed near the amphiaster (Fig. 10). In the dividing egg, they gathered at the equatorinl region. And some of them formed large vacuoles at the middle of the spindle, which had already began to fade (Figs. 11 and 12). They stuck to the cell surface of the cleavage plane until the beginning of the nuclear division, and decreased in number. (Figs. 13 and 14). When the furrowing of the second cleavage began, the vacuoles were visible again only on the second cleavage plane,



Figs 10—15. Sections of eggs of *Strongylocentrotus pulcherrimus* stained with the silver method, showing the behavior of the vacuoles in relation to the cell surface formation. Figs. 10, 11 and 12. Silver-stained vacuoles move to the equator of the egg as the cleavage furrow advances, and form large vacuoles at the equatorial region. Figs 13 and 14. The vacuoles attaching to the cell surface of the equator disappeared gradually in the course of the formation of the new cell surface Fig. 15. The vacuoles are visible mainly on the second cleavage plane, but few on the first cleavage plane.



whereas they were absent on the first cleavage plane (Fig. 15).

The writer is inclined to think that those phenomena in *Strongylocentrolus* is indistinguishable from the behavior of the basophilic vacuoles in *Temnopleurus*. And those vacuoles apparently supply some substance taking part in the formation of the new cell surface and also in the formation of segmentation cavity.

MODE OF SHRINKAGE OF THE EGG IN HYPERTONIC SEA WATER AT THE FIRST CLEAVAGE STAGE

It was noted in the preceding chapters that the new cell surface at the cleavage plane will be formed in relation to the materials of the basophilic vacuoles as well as the silver-stained vacuoles. The presence of the diastema was also described in the egg of *Temnopleurus*. In the present chapter the plasmolysis experiment was carried out for the purpose of making out the local difference of density of the cytoplasm during the course of cleavage.

The eggs of *Strongylocentrotus nudus* were put into hypertonic sea water made by adding 6g. NaCl to 100 cc sea water. The mode of shrinkage was different according to the cleavage stage. The fertilized egg prior to the first cleavage shrank from many sides and became polyhedral. If the eggs of just prior to the first cleavage were put into hypertonic sea water, shrinkage continued mainly at the equator, and as the result, the egg became dumbbell shaped (Fig. 16). The eggs dehydrated at the beginning of furrowing, also,

Figs. 16–20. Shrinkage of eggs of *S. nudus* in hypertonic sea water Fertilization membrane was not drawn in those figures. Fig. 16. Egg placed in hypertonic sea water just prior to the beginning of the furrowing The egg shrank at the equator, where the diastema is already present. Fig. 17. Egg at the beginning of farrowing. Shrinkage is violent at the equator whereas it is not at the pole as well as at the margin. Figs. 18 and 19. Eggs placed in hypertonic sea water after the formation of the new cell surface. The shrinkage proceeds on the side of the cleavage plane. Two concave hemispheres with the inner sides facing each other were formed. Fig. 20. Shrinkage of egg at the beginning of the second cleavage.

shrank at the equator, and so, the majority of the cytoplasm went toward the polar regions; and a long cytoplasmic bridge remained at the center (Fig. 17). After the completion of the furrow the eggs shrank on the side of the cleavage plane, so as to form two concave hemispheres with the inner sides facing each other (Figs. 18 and 19). At the beginning of the second cleavage those hemispheres shrank at the equator of the second division (Fig. 20).

In short, the shrinkage by dehydration occured always at the equatorial region of the dividing cell. This reveals that the rigidity of the superficial portion of the cytoplasm differs remarkably according to the regions; that is, the cell surface of the cleavage plane is feeble in comparison with the polar as well as the marginal region of the blastomere. And, the last mentioned region seemed to be very rigid as if it were a solid flange. And from the observation on the eggs just prior to the beginning of furrowing, the presence of soft cytoplasm at the equator, diastema, was also ascertained.

DISCUSSION.

It was already noted by CONKLIN (1902) that even before the furrow actually appears the future cleavage plane is clearly foreshadowed by the diastema in the eggs of *Crepidula*. The same phenomenon was also observed by TAHARA (1915, 1921) and by CASTETTER (1925) in pollen-mother cells. From the observation of dehydration it was shown that the diastema is already present prior to the beginning of the furrowing. And the basophilic vacuoles as well as the silver-stained vacuoles were observed in the diastema of the eggs of the sea urchins. The behavior of those vacuoles reveals the possibility that they will supply the materials for the new cell surface. And accordingly, the rôle of the diastema during the cleavage will be not only to make the pathway of the furrow but also to supply the materials of the new cell surface.

According to FRY (1937) the mid body of animal eggs is homologous with the cell plate in plant cells. The mid-body of the sea urchins' eggs remained for a while without change, and it showed no sign of forming the cell surface. The phenomena of formation of the new cell surface, which have already been proved by an indirect methed in *S. pulcherrimus* and *Temnopleurus* by the writer (MOTOMURA 1935 and 1940), will depend on the substance other than that of the mid-body.

In the eggs of *S. pulcherrimus* the cleavage advances only by the extension of the egg surface in Ca-free sea water; but if Ca ion is present in the medium a new cell surface is formed at the cleavage plane (MOTOMURA 1941). It will be shown by those facts that Ca ion is one of the important factors for the cell surface formation in the cleavage However, in regard to the chemical relation between the above mentioned vacuoles and Ca ion, further observation will be necessary.

SUMMARY.

Morphological observations during the cleavage of the eggs of the sea urchins, *Temnopleurus hardwickii*, *Strongylocentrotus nudus* and *Strongylocentrotus pulcherrimus*, were carried out.

Special vacuoles adhered by the basophilic granules appearing at the cleavage stage of the eggs of *Temnopleurus*. And, silver-stained vacuoles were observed in the eggs ot *Strongylocentrotus* instead of the basophilic vacuoles in *Temnopleurus*.

The behavior of those vacuoles in both species were alike in the course of cleavge, and it is probable that they will be the material of the new cell surface.

The eggs of cleavage stage shrink at the equatorial region, when they were put into hypertonic sea water. This shows that the diastema is actually present in the sea urchin's egg, and that it is feeble cytoplasm against dehydration in comparison with that of the polar as well as the marginal regions.

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AN IMPROVED METHOD OF ARTIFICIAL INSEMINATION ON SOME MARINE EGGS.[1]

BY

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In this paper is summarized the initial results from the experiments, attempted on studying a special relation of calcium to the activation of unfertilized eggs and the mechanism of sperm entrance in fertilization reactions. And, the appropriate treatments of the fully grown ovarian eggs with the calcium-free sea-water, prior to insemination, will be reported as an improved method of artificial fertilization of the eggs of some marine invertebrates.

MATERIALS AND METHODS

The normal (or homologous) fertilization of the three species of marine invertebrates, and the cross fertilization between two different sea-urchin species were tested. The material, used in the former case, were a clam, *Mactra sachalinensis*, a starfish, *Asterias amurensis*, and a sea-urchin, *Strongylocentrotus nudus*. The materials, used in the latter case, were the eggs of *Strongylocentrotus pulcherrimus* and the sperm of *Strongylocentrotus intermedius*.

The eggs were firstly dissected out of the female gonad, kept without any contamination with sea-water, and quickly released into a dish containing calcium-free artificial sea-water. Thus, the eggs were exposed for varying periods of time to the calcium-free medium in the dish. After this calcium-free bath, the eggs were concentrated towards the center of the dish by gentle rotation, and about 2 cc. of such a dense suspension of the treated eggs were removed through a pipette and transferred to another dish, containing 30 cc. of the standard artificial sea-water. Nearly all of the supernatant fluid in the second dish were also pipetted out quickly and replaced by a fresh standard solution. In this manner, the eggs were washed at least once or twice and recovered from the calcium-free bath. Subsequently, they were inseminated artificially in this recovering condition.

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[1] Contributions from the Marine Biological Station, Asamushi, Aomori Ken. No. 179.

The spermatozoa were not treated with the calcium-free bath. The "dry" sperm, was diluted to 1 : 200 with the standard solution. A few drops of such spermatozoa suspensions were added to the recovering eggs for insemination.

As the controls, the eggs were not treated with the calcium-free medium, but kept in the standard solution for the corresponding periods of time to the calcium-free bath. In this case, also, the eggs were transferred to the second dish of fresh standard solution, and essentially the same insemination method was employed as that of the experimental treatments. The eggs from a single female and the sperm of one male were used invariably for any given comparison.

At each point of investigation, the contents of the dish, experimental as well as control, were stirred by drawing in and out of a pipette. Samples of a few drops, selected at random, were examined repeatedly. Several hundreds of eggs were counted in total to determine the percentage of the developed eggs. No special attempt was made to control the temperature of the experimental solutions, besides the inseminated dishes were kept cool in a stream of the running tap water, in which the fluctuation of temperature was within 3° C. The solutions were mixed in the proportion as shown in Table 1, which has approximately the normal concentration of the cations in natural sea-water.

Table 1. Composition of the artificial sea-water.

Individual salt solution	Standard solution	Calcium-free solution
Distilled water	387 cc	387 cc
1 M $NaCl$	438 cc	438 cc
0.5 M KCl	18 cc	18 cc
0.37 M $CaCl_2$	27 cc	
0.37 M $MgCl_2$	42 cc	42 cc
0.37 M $MgSO_4$	80 cc	80 cc
0.5 M $NaHCO_3$	8 cc	8 cc
Total volume	1000 cc	973 cc

The pH of both solutions was adjusted, by addition of 0.1 N $NaOH$ or 0.1 N HCl, so as to lie in the range of natural sea-water (*i.e.* pH 8.1 to 8.3).

RESULTS

1) *Mactra sachalinensis*

In most cases of *Mactra sachalinensis*, the percentage of the cleaved eggs was lower than 30% by the usual method of artificial insemination (Conts. 1, 2 & 3 of Table 2). When the eggs were treated with calcium-free and recovering baths, a significantly high percentage was obtained as shown in Exps. 1, 2 and 3 of Table 2. The time factors concerning these treatments, both calcium-free and recovering baths, appeared to be very important. In this case, the best results were obtained for the exposure of 60 minutes to the calcium free bath and for the stay of 60 – 120 minutes at the recovering.

Table 2. Improvement of the artificial fertilization on *Mactra sachalinensis*.

No. of experiments	Date and temperature	Numbers of eggs investigated	Treatments prior to insemination		Range of cleaved percent. estimated (α=0.1)
			Ca-free bath	Recovering bath	
Exp. 1	May 10th. 1945 12~10°C	220	60 min.	120 min.	51.6~35.2%
Cont. 1		100	—	180 min.	12.5~ 0.2%
Exp. 2	May 11th. 1945. 12~10°C	242	60 min	120 min	64.2~47.8%
Cont. 2		250	—	180 min	32.7~19.3%
Exp. 3	May 2nd, 1946 15~12°C	301	60 min.	60 min	70.6~64.0%
Cont. 3		306	—	120 min.	29.1~22.5%

2) *Asterias amurensis*

The eggs of *Asterias amurensis*, in good condition, gave generally a little high percentage of the cleaved eggs upon usual insemination (70 – 30%). Moreover, the percentage of these developed eggs showed a great variability, due to the different periods of time for the standard solution, *i.e.* the factor concerning the aging of shed eggs into sea-water. In one batch of eggs from a single female (used in Conts. 1, 2 & 3 of Table 3), the highest percentage of cleaved eggs was shown for the period of 30 minutes of aging; while, in another batch from a different female (used in Cont. A of Table 3), the same high percentage

Table 3. Improvement of the artificial fertilization on *Asterias amurensis*.

No. of experiments	Date and temperature	Numbers of eggs investigated	Treatments prior to insemination		Range of cleaved percent estimated (α=0.1)
			Ca-free bath	Recovering bath	
Exp. 1	April 5th, 1946. 13~10°C	789	15 min.	5 min.	87.8~83.0%
Cont. 1		554	—	20 min.	50.2~43.9%
Exp. 2		583	30 min	5 min	88.5~81.1%
Cont. 2		349	—	35 min.	73.5~62.8%
Exp. 3		293	60 min.	5 min.	87.4~73.6%
Cont. 3		525	—	65 min.	38.7~30.2%
Exp. A	April 7th, 1946. 12~10°C	208	180 min.	5 min.	84.8~78.8%
Cont. A		198	—	185 min.	72.0~62.4%

was found after a period of 3 hours of aging. Such an individual variability for the aging processes may depend upon the degree of cytoplasmic ripeness in different individuals. The former eggs are likely to be more over-ripe than the latter. In either case, however, a constantly improved high percentage becomes evident by the appropriate exposure to the calcium-free and recovering baths before insemination. In the eggs of *Asterias amurensis*, also, the time factors concerning the treatments are very important. The best results were obtained through an extremely short stay at the recovering bath (within 5 minutes), after very long exposure to the calcium-free medium (15 - 180 minutes).

3) *Strongylocentrotus nudus*

On the eggs of *Strongylocentrotus nudus*, a high percentage of fertilization is usually obtained from not treated eggs (100 – 90%). But, in this experiment, eggs from some special specimens of low fertilization-capacity were selected, although the factors abolishing their fertilization-capacity were quite unknown. The influence of the calcium-free bath on the sea-urchin eggs becomes first evident only in such a special case. The two data indicated in Table 4 were such examples, in which, a failed percentage of the control eggs was probably due to the over-ripeness of eggs near the close of the breeding season. In these examples, the appropriate treatments with calcium-free and recovering baths were very effective and produced an exceedingly high percentage of cleaved eggs and improved the failed percentage of the controls. Concerning the available range for the periods of time of the treatments, the results are somewhat similar to the case of *Asterias amurensis* —— an extremely short recovering bath (within 5 minutes), after very long exposure to the calcium free medium (90 – 150 minutes).

Table 4. Improvement of the artificial fertilization in *Strongylocentrotus nudus*.

No. of experiments	Date and temperature	Numbers of eggs investigated	Treatments prior to insemination		Range of cleaved percent estimated ($\alpha = 0.1$)
			Ca-free bath	Recovering bath	
Exp. 1	Oct. 21st, 1946. 18~16°C	103	90 min.	5 min	99.2~92.7%
Cont. 1		114	—	95 min.	57.2~40.6%
Exp. A	Oct. 22nd. 1946. 18~15°C	277	150 min.	5 min.	97.8~95.5%
Cont. A		378	—	155 min	33.5~25.1%

4) ***Strongylocentrotus pulcherrimus* (♀) × *Strongylocentrotus intermedius* (♂)**

It can be seen that the capacity of not treated eggs for cross fertilization gave only a low percentage of normal blastulae (Cont. 1 in Table 5). When the eggs were treated with calcium-free and recovering baths, the cross fertilization-promoting influence became evident through these treatments. Significantly higher cross fertilization-values were, thus, obtained for the present combination, although the percentage of the improved cross fertilization was still slightly below the values obtained for the normal (or homologous) fertilization of each species (Exp. 1 in Table 5). The periods of time necessary for improvement are also indicated by combining long exposure to calcium-free medium (180 minutes) with extremely short recovering bath (5 minutes).

Table 5. Improvement of the cross fertilization in the combination *Strongylocentrotus pulcherrimus* (♀) × *Strongylocentrotus intermedius*(♂).

No. of experiments	Date and temperature	Numbers of eggs investigated	Treatments prior to insemination		Range of cleaved percent. estimated (a [illegible])
			Ca-free bath	Recovering bath	
Exp. 1	April 8th 1946. 13°~10°C	510	180 min.	5 min	71.6~[illegible]%
Cont. 1		485	.	185 min	4[illegible]~2[illegible].1%

COMMENTS ON THE SYNERGISTIC ASPECT OF ACTIVATION BETWEEN THE PREVIOUS BATH IN CALCIUM-FREE MEDIUM AND THE REMOVAL TO SEA-WATER

It is well known that the calcium ion in the fertilization-medium plays an important rôle upon artificial insemination of many marine eggs. In many authorized investigations reported already (*cf.* HOBSON 1928, SCHECHTER 1937 & '41, HOLLINGSWORTH 1941, *etc.*), and also in my provisional trials, the following two aspects are distinguished from this kind of actions of the calcium firstly, the presence of the calcium in the medium is presumed to be essential at least for the sperm entrance and the membrane formation of fertilization reactions, and also the eggs can be never fertilized in the calcium-free medium itself; secondly the life span of unfertilized eggs is prolonged by the storage in the sea-water of low calcium contents. Through the data reported here by myself, a third aspect may be added to the action of calcium in question. In this third aspect, it is noteworthy that distinct improvement of artificial fertilization results from the synergistic effects between the calcium-free and the recovering baths of the fully grown ovarian eggs. Effects very similar to that of calcium are reported, recently by LEFEVRE (1945), in regard to the actions of picric acid on the activation of unfertilized *Nereis* eggs: *viz.*, when the eggs are activated artificially at the removal from the previously short stay at 0.001 M picric acid sea-water to the ordinary sea-water, the synergistic actions between these procedures cause a clear-cut increase to the response of the nuclear break-down.

There are several theories to explain the activation of unfertilized eggs (f.i. oxidation theory by LOEB, permeability theory by LILLIE, coagulation theory or colloid chemical theory by HEILBRUNN, depolarization theory by DALCQ, calcium release theory by HEILBRUNN and his collaborators, *etc.*). All these theories postulate that the stimulation must involve physicochemical changes within cells. According to the calcium release theory of stimulation (*cf.* HEILBRUNN & WILBUR 1937, WILBUR 1939), it is conceivable that the activator substance is equivalent to the free calcium released from the cell-cortex. The synergistic aspect of activation demonstrated in the present investigation may also be explained by the presence of activator substance suggested in the calcium release theory of stimulation. It is supposed that some unknown physicochemical disturbance, possibly causing the liberation of masked calcium, occur at the egg-cortex by the previous treatment with calcium-free medium. At the removal to the standard solution, the egg-cortex undergoes a series of subsequent changes and may be recovering from disturbance to attain the physiologically stable balance. The condition favouring the sperm entrance appears probably in the course of these changes.

In recent papers, RUNNSTRÖM and MONROY have emphasized the possibility that actions of some proteolytic enzymes or the detergent like Duponol may play a leading rôle in the activation of the eggs (*cf.* RUNNSTRÖM 1948, MONROY & RUNNSTRÖM 1948, MONROY 1948). We can imagine, therefore, that a possible factor activating a chain of these enzymatic reactions may be awaken in the processes of recovery from calcium-free bath. In fact, the egg-cortex seems to attain the stable balance in excess of recovering bath, because the effectiveness of treatment is missed again by the insemination practised after too long a stay in the standard solution.

In the present experiments, no good result is obtained, unless the fully grown ovarian eggs are directly treated with calcium-free medium and recovered appropriately in standard solution. The improvement of fertilization is always obscured by means of treatments of the shed eggs into the sea-water. Moreover, the treating periods of time, available for the improvement of fertilization, vary in the eggs of different forms and individuals as stated already. It is suggested that the most appropriate periods of time treated with calcium-free bath are rather short for the molluscan egg and somewhat long for the echinoderm eggs. The best recovering periods are, on the contrary, remarkably long for the mollusc and extremely short for the echinoderms. Besides the limited range of appropriate treatments for each species, the percentage of the cleaved eggs has a tendency to be rather below the values obtained for the controls. In every case of the present experiments, no development of the eggs took place by the mere removal to standard solution itself, even if the eggs were exposed much longer to calcium-free medium. In addition, some indications of the polyspermatic eggs or the abnormally loosened segmentations were observed in a few samples of *Mactra* eggs, which were tested by inseminating too soon after removal to the standard solution. These samples were, however, too few to justify any conclusion. At all events, it is remarkable that the effectiveness of the treatments becomes first evident, when the synergistic influences are appropriately utilized between the previous calcium free bath and the subsequent recovering to sea-water.

SUMMARY

The artificial fertilization of some marine invertebrates is significantly improved by means of newly deviced treatments, in which the fully grown ovarian eggs, exposed to the calcium-free sea-water previously, are recovered by transferring to the artificial sea-water, and subsequently inseminated there by the sperm. By this method, good results are obtained for the fertilization of *Mactra sachalinensis*, *Asterias amurensis*, and *Strongylocentrotus nudus*. and also for the cross fertilization between the eggs of *Strongylocentrotus pulcherrimus* and the sperm of *Strongylocentrotus intermedius*.

The time factors available for the effective treatments, both the calcium-free and the recovering baths, are very important and variable in the eggs of different species and individuals. The distinct improvement of fertilization becomes evident only through the synergistic influences, where the treating periods of time for the calcium-free and the recovering baths are appropriately combined with each other.

It is supposed that some physicochemical changes favouring the sperm entrance are awaken in the egg-cortex of recovering processes from the previous calcium-free bath. In this respect, the research will be continued both from the physiological and the morphological points of view.

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STUDIES ON THE CONJUGATION OF *PARAMECIUM CAUDATUM*. III. SOME PROPERTIES OF THE MATING TYPE SUBSTANCES.[*]

BY

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(With 4 Text-figures)

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INTRODUCTION

It has been shown in the previous paper (HIWATASHI. 1949a) that the formalin-killed animals of a certain mating type clump with living animals of the opposite type and subsequently yield selfing pairs in the living type. In addition to this, he reported that in the reciprocal combinations of living and killed types, in which the living animals of types 1, 3, 6 and 7 were mixed with killed animals of types 2, 3, 5 and 8 respectively, no clumping between the killed and living animals were observed.

In relation to those facts the author carried out the following two experiments; that is, inactivation of the formalin killed reactive animals by treating with some agents, and further studies on the failure of the reaction in the reciprocal combinations mentioned above.

The writer wishes to express his hearty thanks to Prof. ISAO MOTOMURA for his supervision throughout this work.

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MATERIAL AND METHOD

Four stocks of *Paramecium caudatum* were used for the present experiments; they are $L_B 5$ (type 1), $L_B 6$ (type 2), $K_A 6$ (type 3) and $K_3 2$ (type 4), the former two belong to group 1 and the latter two to group 2 respectively in the writer's collections. The same method of culture described in the preceding paper (HIWATASHI, 1949a) were mostly used, and in some experiments, 0.3% cold extract of clover leaves with a small quantity of dried yeast was also used. In order to induce the maturation for mating, rapidly multiplying paramecia were washed with sterile tap water and concentrated to the density of about 4,000 individuals in 1cc. of fluid. Seven hours or more after this procedure, a strong mating capacity was induced. Cultures of animals and tests of mating reaction were carried out at 25°C.

RESULTS.

TREATMENT OF FORMALIN-KILLED ANIMALS WITH SOME AGENTS

It was noted in the previous paper (HIWATASHI, 1949b) that formalin-killed animals of a certain mating type react strongly and specifically with living animals of the opposite type. In the present experiments, the conditions of the reactivity in the formalin-killed animals were tested by treating them with some agents. Chemical agents used were ethanol (95%, 75% and 25%), methanol (80%, 40% and 20%), acetone (95%, 50% and 10%), acetic acid (5% and 1%), urea (from 30% to 1%) and ammonium sulfate (from saturated to 5%).

In the first experiment, the mating-active animals of type 3 were killed by 3% formalin and washed thoroughly with tap water. As stated above, the killed bodies still retain their specific reactivity. Immediately after this procedure, the killed bodies were treated for 5 or 10 minutes with one of the chemical agents mentioned above at room temperature (18°-20°C), and washed again with tap water. They are, then, mixed with the living animals of type 4 As the results, when the killed bodies were treated with one of the agents, 25% ethanol, 20% methanol, 10% acetone, 15%-10% urea or ammonium sulfate from saturated solution to 5%, the mating reaction and the following selfing induction occurred in the mixtures. And, on the other hand, the reactivity of the killed-bodies of type 3 was inactivated, when they were treated with acetic acid, or with ethanol in 95% and 75%, methanol in 80% and 40%, acetone in 95% and 50% or urea from 20% to 30%.

In the second experiment, animals of type 3 were killed by one of those solutions ineffective to the reactivity without previous treatment with formalin. When the killed bodies were mixed with the living type 4, the clumping between the types were observed. The bodies killed by ammonium sulfate solutions of various concentrations showed the best result, the strong specific clumping and subsequently induced selfing. And the mating reaction as well as the selfing induction were poor, when the bodies were killed by one of the solutions; 25% ethanol, 20% methanol, 10% acetone and 15%-10% urea. It seems to be probable that the nature of the killing action of ammonium sulfate will be the dehydration. And, therefore, glycerine was tested, which is one of the dehydration agents. Animals were killed by glycerine in concentrations from 50% to 5% and were mixed with the living animals of the opposite type. As it was expected, the result was positive in the concentrations over 20%, but in the cases of low concentrations, the killed animals cytolysed and the reactions were negative.

Another set of experiments on the inhibition of mating reactivity were carried out by heat and various pH values. Formalin-killed animals heated to 45°C. for 5 minutes in tap water retained their specific reactivity to living animals of the opposite type, but they failed to react after 5 minute exposure at 50°C. In the pH experiments, mating reactive animals killed by formalin were treated with tap water of various pH's, from pH 2.5 to pH 10.0. No buffer solution was used and the change of pH of the solutions measured about 0.2 after 30 minutes from the beginning of the experiments. Formalin-killed animals retained their specific reactivity after 30 minute treatment at 25°C. with water, acid to pH 5.0 or basic to pH 9.2. No reactivity was left when treated with water more acid than pH 4.8 or more basic than pH 9.5. Thus the pH stability range of the mating reactivity of killed animals showed a clear-cut result.

Finally, the formalin-killed reactive animals were disrupted completely by grinding and were then centrifuged. Neither the centrifuged solid part nor the supernatant fluid had any mating effect on the living animals.

DIFFERENCE OF THE ACTIONS OF KILLING AGENTS ON THE MATING TYPES.

In the previous paper (HIWATASHI, 1949b), the writer pointed out that, in the reciprocal combinations of killed and living types, killed animals failed to react on living animals of the opposite type. In the present study, this phenomenon was analysed under various conditions of the concentration of killing agents, the kind of killing agent, and time of treatment. As shown in Figs. 1 and 2, formalin-killed type 4 or type 2 loses its reactivity after the treatment for a few minutes with formalin even in low concentrations, whereas in types 1 and 3, the inactivating effect of formalin was slow in the low concentrations.

Here, the cause of the failure of mating reactton in the reciprocal combina-

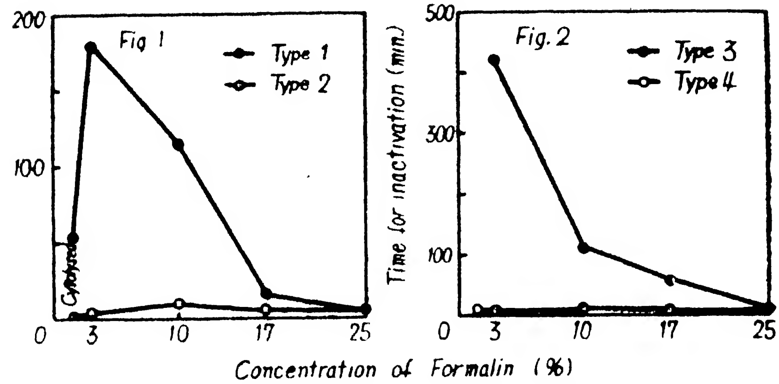


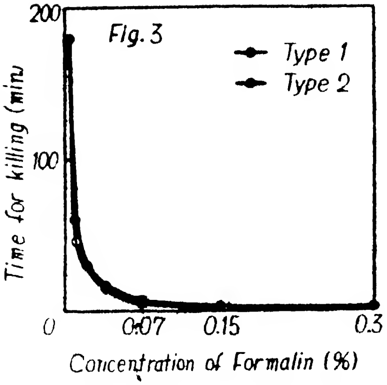
Fig 1 Relation between concentration of formalin and time for inactivation of mating reactivity in complementary types of Group 1.(12°C)

Fig. 2 Same relation as Fig. 1 in complementary types of Group 2.(20°C)

tions of killed and living types, described in the previous paper, can be interpreted as the remarkable difference of the effect of formalin between two types of the same group. Furthermore, those figures show that the mode of the inactivation of mating reactivity by formalin is unlike in two complementary types of a group. Such an analysis was not carried out in groups 3 and 4. It will be possible, however, that a similar relation will be found also in those groups, because the mating reaction between the killed and living types was observed in the cases of 20 minute treatment with 5% formalin of types 6 and 7, and mixing with the living types 5 and 8 respectively, but in the reciprocal combinations it was not the case. Thus the mating type difference of the mode of inactivation by formalin seems to occur in all the four of the Japanese groups (varieties) of *Paramecium caudatum*.

Analytical experiments whether the above mentioned difference is due to the fixing action of formalin or to the action of its acidity, were carried out in the following way; that is, to compare the relation of time and concentration of formalin necessary for killing the animals, and next the effect of pH of the killing agent on both types. Experiments showed that there is no difference between the tendencies of killing action of formalin in both complementary types (Fig. 3). Values of pH were about 5.2 in 25% formalin and 5.6 in 5%. And when 50% glycerine acidified with HCl was used as the killing agent, the critical pH for inactivation of the mating reactivity measured equally 5.0 in both complementary types (Fig. 4).

Another sorts of experiments concerning the same problem, were performed using ammonium sulfate and glycerine as the killing agents. The effect of



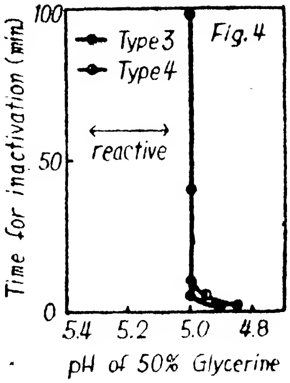


Fig. 3 Relation between concentration of formalin and time necessary for killing paramecia in complementary types of Group 1. (20°C)

Fig. 4 Relation between acidity of glycerine and time necessary for inactivation of mating roactivity in types of Group 2. (20°C)

ammonium sulfate was similar to glycerine but was different from formalin. It is noteworthy that in the case of glycerine as well as ammonium sulfate, the mating reactivity was equally retained by short treatment, for example 40-60 minutes. In some cases, the difference between two types of the same group was observed when the animals were preserved for a long time in the solution. Animals of type 1 lost their reactivity in a saturated solution of ammonium sulfate after 12 hours at 13°C, while animals of type 2 retained their mating capacity for 10 days. In 50% glycerine animals of type 1 lost their reactivity after 80 minutes, while type 2 was reactive for more than two days. The differences between the types in group 2 were not observed with those two agents, because the mating capacity of types 3 and 4 were equally preserved for a week either in saturated solution of ammonium sulfate or in 50% glycerine.

DISCUSSION

It was reported by many authorities that the mating reactivity of paramecia is inactivated by various agents; they are heat, extreme pH, complete disruption of animals (METZ, 1946) and specific antiserum (METZ and FUSCO, 1948) in *Paramecium aurelia*; X-ray in *P. calkinsi* (WICHTERMAN, 1948a) and in *P. bursaria* (WICHTERMAN, 1948b). According to the present studies on *P. caudatum*, heat, extreme pH, and complete disruption of animals could inactivate the mating reactivity of formalin-killed animals. Besides those agents, some chemical agents were found to inactivate the formalin-killed reactive animals; they are ethanol, methanol, acetone and urea in high concentrations, and acetic acid.

In addition to those results, it was shown in the present experiments that in formalin the mating capacity of a certain mating type was more stable than that of the complementary type of the same group. METZ (1948) reported in his studies on *P. calkinsi* that formalin-killed animals of type I of his species could react to living animals of type II and induced pseudoselfing in the living type, while killed type II failed to react to the living type I. The conditions of the mating reactivity in *P. caudatum* seems to resemble those in *P. calkinsi*. In *P. aurelia*, such a difference of the stability of mating capacity between mating types has not been reported so far as is known to the writer.

Further, in the present study, it was found that ammonium sulfate and glycerine are good killing agents for preserving the mating reactivity. The remarkable difference of stability of mating reactivity found in the formalin-killed animals of complementary types were not observed in the animals killed either by ammonium sulfate or by glycerine. In this respect, the nature of those two agents must be distinguished from that of formalin.

SUMMARY

Investigations on the properties of mating type substances in *Paramecium caudatum* were carried out. The results were as outlined below.

1) The mating reactivity of formalin-killed paramecia was inhibited by heat, extreme pH, complete disruption of killed animals, ethanol, methanol, acetone, acetic acid or urea.

2) The mode of inactivation of the mating reactivity by formalin was unlike in both complementary types of a group.

3) Ammonium sulfate and glycerine were found to be good killing agents differing from formalin in the point that the former two retain their mating reactivity of dead animals equally in both types of a group.

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A CULTURE METHOD OF THE EGGS OF A TERRESTRIAL ISOPOD, *ARMADILLIDIUM VULGARE* (LATREILLE).*

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The present paper deals with the results of the tests on the culture methods of the eggs of a terrestrial isopod, *Armadillidium vulgare* (LATREILLE), for the purpose of embryological study. The culture methods of the eggs of crustaceans have been reported by many authorities. In many species of cladocera and *Succulina*, the eggs can easily be cultured in the artificial media without adding any organic nutritive substances (RAMMNER, 1933; RAMULT, 1935). On the other han ind *Moina* and others, according to RAMMNER (1933), it is difficult to culture the eggs outside the brood-pouch. And in relation to the eggs of the terrestrial isopod the field is as yet untouched, as far as the writer is aware. Recently the writer took note of the interesting relations of Ca ions for the normal development of the eggs of *Armadillidium*. The writer wishes to express her sincere thanks to Prof. Dr. ISAO MOTOMURA for his supervision during the course of this work.

MATERIAL

The material used was *Armadillidium vulgare* (LATREILLE) collected from the neighbourhood of this laboratory. In the natural condition, the eggs are kept in the mother's brood-pouch until the larvae go out. Each female contains about 50 to 100 eggs in the brood-pouch. For the experiment, the eggs of the

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brood-pouch were taken out in the early stage of development before the formation of the blastoderm, and were cultured in the artificial media.

RESULTS

In the first experiments the effects of three kinds of salt solutions on the developing eggs were tested. They are sea water, SPEK's solution made of 6 g. NaCl, 0.2 g. KCl, 0.2 g. $CaCl_2$ and 0.2 g. $NaHCO_3$ per litre of water, and VAN HARREVELD's solution made of 12 g. NaCl, 0.4 g. KCl, 1.5 g. $CaCl_2$, 0.25 g. $MgCl_2$, and 0.2 g. $NaHCO_3$ per litre of water. Various concentrations of those solutions were tested without changing the proportion of the constituents. In the former two media the eggs could not develop, notwithstanding various osmotic pressures of the solutions. And this showed that a special composition is needed for the culture media Fortunately, in VAN HARREVELD's solution which is the physiological solution for the tissues of *Astacus* and *Cambarus*, the eggs showed a tendency of development. And it was shown that the range of the osmotic pressure suitable for the development was relatively wide. That is, in the solutions from 0.36 Mol to 0.72 Mol calculated for the osmotic pressure of the solution of non-electrolyte, over 24 % of the eggs developed to the hatch out stage, whereas in 0.84 Mol only 8 % of them hatched out. The optimum concentration of the VAN HARREVELD's solution is, therefore, 0.48 Mol which is equal to that of the original solution.

As mentioned above, VAN HARREVELD's solution contains a large amount of $CaCl_2$, which is a necessary condition, in comparison with the other two solutions. As shown in Table I the rate of developed eggs was large, when the amount of $CaCl_2$ was 1.6 g. per litre of water. Contrary with this, when the amount of KCl was increased no larva was observed.

The amount of $MgCl_2$ as well as KCl showed no remarkable influence on the development, if the solution contains rich Ca ions (Table II). There was no

Table I

No. of test solution	Salts contents per litre of test solution in grams					Osmotic concentration in Mols*	Duration of life in days	Percentage of hatching
	NaCl	KCl	$CaCl_2$	$MgCl_2$	$NaHCO_3$			
(1)	12	0.4	1.6	0.4	0.2	0.47	12.7	55.0
(2)	12	0.4	0.8	0.2	0.1	0.45	7.7	2.0
(3)	12	0.4	0.8	0.2	0.2	0.46	5.3	0.
(4)	12	0.4	0.8	0.2	0.4	0.45	4.5	0.
(5)	12	1.6	0.4	0.2	0.1	0.47	3.6	0.

* The osmotic concentration was calculated on the basis of complete dissociation of electrolytes and expressed in isosmotic molar concentration of non-electrolytes.

Table II

No. of test solution	Salts contents per litre of test solution in grams					Osmotic Concentration in Mols*	Percentage of hatching
	NaCl	KCl	$CaCl_2$	$MgCl_2$	$NaHCO_2$		
(1)	12	1.6	1.6	1.6	0.2	0.53	63.0
(2)	12	0.4	1.6	0.3	0.2	0.48	55.0
(3)	12	1.6	1.6	0.8	0.2	0.52	52.0
(4)	12	1.6	1.6	0.4	0.2	0.52	51.0
(5)	12	0.8	1.6	0.4	0.2	0.49	50.0
(6)	12	0.4	0.4	1.6	0.2	0.49	0.

*The same as in Table I.

statistically significant difference in the five solutions named (1), (2), (3), (4) and (5) in Table II respectively. But in the case, in which the amount of Ca was decreased but that of Mg was increased, no embryo developed.

It has been reported that REY (1935) cultured the parthenogenetic eggs of *Daphnia* in a modified RINGER's solution. Unfortunately, the literature bearing on REY's original method was inaccessible to the writer.

SUMMARY

The culture method of the eggs of *Armadillidium* outside the brood-pouch was studied. It was ascertained that in the culture medium a relatively large amount of Ca ions is necessary in comparison with the ordinary RINGER's solution. The composition of the medium is tentatively stated as 12 g. NaCl, 1.6 g.KCl, 1.6 g. $CaCl_2$, 1.6 g. $MgCl_2$ and 0.2 g. $NaHCO_3$ per litre of water.

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ENERGETICS OF THE HEART MUSCLE OF THE OYSTER, WORK PERFORMED AND OXYGEN CONSUMPTION[1)2)]

BY

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(Received August 31, 1949)

Numerous studies have been made on the physiology of molluscan hearts, but no or few works have appeared on the energetics of this organ, as far as we are aware. The author attempts in this paper to elucidate, for the first time, the energetic relations between the mechanical activity and metabolic rate of the heart of the oyster, *Ostrea circumpicta* PILS. This species of oyster is a large form and its heart affords a favorable material for the study of this kind. Many studies on the heart of this species have been carried out in our marine laboratory (KOKUBO, NOZAWA, TAKATSUKI, *etc.*). The excised heart can survive 10 days or more in sea-water, and continues pulsation if conditions are favorable. The number of specimens of this species in the vicinity of the laboratory has been decreasing and I had to save the material. Moreover, the manipulation of this small organ is a delicate task and experiments might often fail. The data given below are examples of results of a few successful experiments.

MATERIAL AND METHOD

The specimens used in the present work usually measured about 15 cm. in shell length and are much larger than the ordinary edible oyster in this country,

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1) Contributions from the Marine Biological Station at Asamushi, Aomori Ken. No. 18[illegible].

2) The expense of this work was partly defrayed by a grant from the Scientific Research Expenditure of the Department of Education, to which the author expresses his cordial thanks.

Ostrea gigas THUNBERG. The right shell valve was removed and the pericardium was carefully opened. The procedure of the present work essentially consisted in recording the contraction curve of the heart muscle and in determining the oxygen consumption of the heart muscle during the experimental period. From the contraction curve, the amplitude and frequency of contraction could be determined, and these, with the load on the heart muscle, gave the work performed by the heart muscle. For the present purpose, the ventricle alone was used, as the contraction of the auricle obscures the contraction of the ventricle in the contraction curves. So the ventricle was ligated near the aorta and at the auriculo-ventricular junction, with pieces of thin silk thread, then cuts were made outside the ligatures on both sides, and the ventricle was taken out, and was suspended in a large amount of sea-water until used. The sea-water had been filtered and 20 or 30 cc. of it were taken into each of three small glass vessels. One of these was used for oxygen determination at the beginning of the experiment and the second was used to ascertain that the oxygen content did not change appreciably without the heart muscle. The third was used for the experiment, allowing the heart muscle to respire therein. The difference of oxygen contents of the control and experimental respiratory vessels gave the amount of oxygen consumed by the heart muscle. The latter was connected to the supporting rod in the vessel on the one side and to the lever on the other side. The surface of the respiratory medium was covered with a layer of liquid paraffin 3 cm. thick, to prevent diffusion of oxygen from without. The determination of oxygen was made by micro-WINKLER method with 1/100 N thiosulfate solution. The whole respiratory medium was used for analysis, reagents being poured directly into the vessel after the muscle preparation was taken out. The heart muscle showed some degree of spontaneous movement even when no weight was loaded upon the muscle, but the rhythmic contraction became quite conspicuous and regular and continued for a long period when appropriate weight was loaded. When the muscle heart contracts longitudinally, it expands laterally and *vice versa*. The amplitude and frequency of the contraction of heart muscle depend on the tension of the heart muscle, that is on the amount of blood in the heart and weight of the load. The former factor could not be controlled in the minute organ of the present study. For the estimating the work performed, only the longitudinal contraction of the heart muscle could be taken into consideration. The ventricle did not work as a pumping machine in this case. On the way, however, it may be mentioned that the ventricle showed, in one case, contractions on one side and relaxation on the other side, and these changes circulated around the longitudinal (auriculo-ventricular) axis of the heart.

For calculating the work done, the general formula for the heart work can not be applied in this case, and the general formula for skeletal muscle should be employed, which is as follows:

$$W = lh + \frac{1}{2} mh$$

where W is the work done, l is the load, and h is the height the load is lifted. m is the weight of the muscle, but in practice in this case, only the difference of the specific gravity of the muscle and the sea-water is effective and should be taken into account. The specific gravity of the heart muscle is not known, but was assumed to be 1.05 as the specific gravity of protoplasma in general varies between 1.02 and 1.05 (STEMPELL and KOCH 1923). The specific gravity of the sea-water was about 1.024. The weight of the heart muscle was 0.092 grams (Exper. 9, Heart No. 3) and this was multiplied by the difference of specific gravities (1.05 -1.024 = 0.026).

The above formula applies to the work done in a single twitch of skeletal muscle. The work of the heart muscle required in this study is the sum of work performed by a series of contractions, which compose the rhythmic pulsation of the ventricle. The term $(l + \frac{1}{2} m)$ does not change throughout each experiment, but the height or amplitude and frequency of contraction varied slightly. So the curve of contraction was divided into many parts, where the amplitude and frequency remained almost unchanged. The amplitude and frequency of representative portion of each part were determined, and (frequency $\cdot$ t) gave the number of contractions of each part and (amplitude × number of contractions) gave the total height the load was lifted in each part of the contraction curve. The muscle preparation was tied to the lever 6 cm. apart from the fulcrum, and the writing point of the lever was 20 cm. distant from the fulcrum, so the amplitude in the curve was multiplied by 6/20 to obtain the true amplitude. From the data above mentioned, the work done in each part of the contraction curve could be calculated, and the sum of the partial work thus obtained gave the total work performed during the experiment. The calculation was simple, although laborious, and the details are omitted and the results are given in the Table.

RESULTS OF EXPERIMENT

Acting and resting oxygen consumption The results given in table raise various problems. The rate of oxygen consumption per unit weight and per unit time varies considerably and is not always higher when the heart muscle is loaded than when it is unloaded, even in the same preparation. Such irregularities are also met with in other animals (CLARK 1938). The cause of this is not error in oxygen determination. The figures in the Table, however, give us an idea of the order of magnitude of the rate of oxygen consumption in the heart muscle of the oyster as compared with that of other tissues or animals. In the vertebrate skeletal muscle, the resting oxygen consumption is 0.003 cc. O_2 per g. per min. (= 180 cmm. per. g. per hr.) and the acting muscle uses oxygen 0.03 cc. per g. per min. (= 1800 cmm. per g. per hr.) The increase due to activity is, therefore, ten times the resting rate. The figures in Exper. 2 are doubtful and may better be discarded. The increase in the oyster heart muscle does not exceed 50 % of the resting oxygen uptake. This may probably be due to the fact that the heart muscle of the oyster shows spontaneous movement even when it is not loaded, and the "resting" respiration is not true resting respiration in this case, as has been mentioned above. The resting respiratory rate would reasonably be expected to be high.

Work performed and mechanical efficiency. If Experiment 9 a and b would represent the acting and resting oxygen consumption respectively, the work performed and the mechanical efficiency of the oyster heart muscle could be estimated. The load was 0.1463 grams and the weight of the muscle 0.092 grams.

Table 1

No. of Exper.	No. of Heart	Weight of heart	Temp. °C.	O_2, cmm consumed	O_2 consumed cmm./g/hr	Period of exp	Condition*
1	1	0.127	[illegible]	[illegible]	51.56	4h [illegible] m	L
2	1	〃		21.28	45.66	3 40	〃
3	1	〃	21	[illegible]	[illegible]	6 00	〃
4	1	〃	21	135.52	63.38	16 50	R
8	3	0.107		14.84	81.54	1 42	L
9a	4	0.092	24.6	**28.00**	**87.78**	3 28	L
9b	4	〃	23.8	**24.92**	**60.92**	4 30	R
10	5	0.078	[illegible]	21.28	74.41	3 40	L

* L=loaded, R=resting

The weight of the muscle in excess of the weight of sea-water of the same volume was only 0.002372 g. The total work performed is given as follows:

$$\text{Total Work} = \Sigma\,(\text{partial work}) = \Sigma\,(l + \frac{1}{2}m)\,h = (0.1464 + 0.001186) \times \Sigma h$$

$$\text{and} \qquad \Sigma h = \Sigma(AFt)$$

where A is the amplitude, F the frequency and t the time of each period. Ft gives the number of contractions in each period, and $h = AFt$ gives the total height the load was lifted in each period.

The calculation gave the value of the total work to be 223.16 g. cm., and the work per g. per hour is 701.0 g. cm.

KOBAYASHI determined glycogen and lactic acid content of the adductor muscle of this animal, but we have no data bearing upon the metabolism of the heart muscle contraction in this organism. If we assume, however, that the energy source of heart muscle contraction is glycogen as in vertebrate, and oxidation of glycogen using 1 L. of oxygen yields 5.14 Cal., then the oxygen usage in doing work in excess of the resting oxygen uptake can be converted in energy units, and this amount of energy should have been expended in the performance of the work. The heart muscle used 26.86 cmm. oxygen per g. per hour, when worked, in excess of the resting usage. 0.092 g. of tissue used in 3 hr. 28 min. therefore, $26.86 \times 0.092 \times 3.46 = 8.55$ cmm. of oxygen, in excess. One litre of oxygen in glycogen oxidation yields 5.14 Cal. and 1 Cal is equivalent to 425 Kg-m. of work, the above volume of oxygen, 8.55 cmm., corresponds to $8.55 \times 5.14 \times 425 = 1867.7$ gram-centimers of work. The mechanical work done, calculated before, is 223.16 g-cm. The mechanical efficiency of the heart muscle of the oyster is therefore

$$\text{Mechanical Efficiency} = \frac{\text{Work performed}}{\text{Energy expended}} = \frac{223.16}{1867.7} \fallingdotseq 0.119$$

DISCUSSION

The data presented here are not abundant owing to scanty of the material and difficulties of experiment, but the results seem to be quite significant as the knowledge in this line is poor at present. The rates of oxygen uptake of the allied forms given by previous authors in books and papers are as follows: —

Species	*Oxygen uptake, cmm./g/hr.*	*Authority*
Ostrea sp.	13.75	ROGERS
Ostrea edulis	15	HEILBRUNN
Ostrea circumpicta	10.2 (22°C.)	NOZAWA
〃 〃	13.5 (25°C.)	〃
Ostrea gigas	52 ~ 246	ISHIDA

It is comprehensible that these values, except in *O. gigas*, are lower than the values obtained in the present study, as the heart muscle would be more active than the other tissues of the oyster.

In vertebrate skeletal muscle, performance of work increases the rate of oxygen uptake by 10 times the resting rate, while the nerve consumes 10.8 cmm. and 26 cmm. per g. per hr. in resting and excited state respectively. A frog's heart under conditions of moderate activity uses about 1 cc. of oxygen per g. per. hr. (CLARK 1938. p. 58). CLARK and WHITE failed to show any increased oxygen consumption by the frog's ventricle or auricle by increasing the work performed. They found, however, that under isotonic conditions the oxygen use was 1.8 cc. per g. per hr. and that this rose to 3.2 cc. under conditions that were nearly isometric (CLARK *loc. cit.*, p. 86). "Recent advances in our knowledge of cardiac physiology suggest that much of the evidence relating metabolic rate and work is inconclusive. Cardiac tissue, when well supplied with oxygen, oxidises lactates but oxidises little or no carbohydrates, but any shortage in oxygen supply causes glycolysis. Any interference with the irrigation of the frog's ventricle is therefore more likely to cause an increased glycogen or sugar usage (by glycolysis) than to cause an increased oxygen usage."

OKAZAKI and KOBAYASHI determined the glycogen content of the oyster, *Ostrea circumpicta* and found that the adductor contains only 1.591 %, while the rest of the body contains 4.611 % of the fresh weight. It is well known that mulluscs in general are rich in glycogen and its important rôle in the metabolism may be expected, but we have no concrete knowledge of the cardiac metabolism of the oyster at present. "In the case of the isolated heart of the dog, therefore, the evidence appears to be fairly complete that a large increase in work (*e.g.* five-fold) causes the oxygen consumption to be doubled and that a part of this increase is due to increased oxidation of lactates." (CLARK *loc. cit.*) In the tortoise's auricle, work equivalent of oxygen usage in excess of basal metabolism gives the mechanical efficiency to be 28 to 39 %, according to conditions. In the case of the human body, the mechanical efficiency, under good conditions, may attain a value of 25 %. (HILL 1927). The mechanical efficiency of the heart muscle of the oyster reported here is not far from our expectation, as the lower organisms such as the oyster would not have advanced mechanism, and moreover only the longitudinal contractions were taken into account in calculating the work performed. The calculation of the mechanical efficiency was made on the basis that oxidation of glycogen supplies the energy for contraction. The present study is merely an attempt to correlate the mechanical activity and metabolism of molluscan cardiac tissue, according to the conventional method which has been usually adopted, but the author hopes this may be the first step of approach to the elucidation of the problem. The present status of our knowledge of the problem being such as discussed above, further thoroughgoing studies are required.

SUMMARY

1. The rate of oxygen consumption of the ventricle muscle of the oyster,

Ostrea circumpicta PILS. was determined in resting and loaded conditions. In resting state, the rate was 63.38 and 60.92 cmm. oxygen per g. fresh weight per hour, and in loaded state it varied from 45.66 to 87.78 cmm. oxygen per g. per hour.

2. A heart muscle preparation, weighing 0.092, performed work of 223.16 gram-cm. in 3 hr. 28 min. (= 701.0 g. cm. per g. per hr.)

3. In doing the above mentioned work, energy of 1867.7 gram-cm. was expended, and the mechanical efficiency was 11.9 %.

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PHYSIOLOGICAL STUDIES ON THE PIGMENTARY SYSTEM OF CRUSTACEA.

IV. STUDIES ON THE DIURNAL RHYTHM OF THE EYE PIGMENTS OF THE SHRIMPS.*

BY

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(With 5 figures and 1 plate)

(Received August 31, 1949)

INTRODUCTION

Diurnal rhythm in behavior and physiological rhythms in certain organ systems of animals have long interested biologists and led to much speculation as to the nature of the mechanisms involved in such activities. Out of the many daily changes in animal activities, some would appear to be quite satisfactory for studying. Such periodicity is most strikingly exhibited in the pigmentary changes of various animals, being reported in shrimps at first by GAMBLE and KEEBLE (1900), in brachyurans by MEGUSǍR (1912), in isopods by MENKE (1911), in amphibians by SLOME and HOGBEN (1929), in reptiles by REDFIELD (1918), and in cyclostomes by YOUNG (1935), etc.

WELSH (1930 a, 1935) has shown that diurnal changes in the migration of the retinal pigments occur in several crustaceans even though the animals are maintained under constant conditions of illumination or of darkness. In such a way, WELSH (1930) found that the distal pigment in *Macrobrachum* by day assumes the position characteristic for dark-adaptation, independently of the intensity of

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*) This report is a part of the series of papers submitted to the Faculty of Science, Tôhoku University, in partial fulfilment of requirements of the degree of Dotctor of Science, March 1948.

light. The diurnal independent migration of the retinular (proximal) pigment was found by BENNIT (1932) in *Cambarus* and WELSH (1935) in *Penaeopsis goodei*. Furthermore, in 1935, WELSH detected a diurnal migration of the reflecting pigment cells in *Latreutes fucorum*, *Leander tenuicornis*, and *Leander affinis*, and in 1936 he observed the same phenomenon in both the distal pigment cells and the reflecting pigment cells in *Anchistoides antiguensis*.

The common occurrence of a diurnal rhythm in the movements of the eye pigments in crustacea shows with certainty that the influence of light on the pigment cells is neither direct nor decisive. BENNITT (1929, 1232) suggested already in 1924 at the possibility of a humoral control of these functions, but did not regard it as very important; later he (1932) and PARKER (1932) discussed the same view more seriously. Since WELSH (1930) was able to prevent the diurnal rhythm under constant illumination by ligation of the eye stalk, the humoral hypothesis has been more and more emphasized. Later, the theory of a humoral regulation of the migration of the eye pigments in crustacea was definitely proved by KLEINHOLZ (1934, 1936, 1937, 1938) in *Palaemonetes vulgaris*. His studies (1934) presented evidence for a humoral control of the distal pigment, but the proximal pigment appears not to be affected by the same eye stalk extracts, which activates distal and reflecting pigments. This seems to be the case also with a shrimp *Paratya compressa* in my own expeniment (1947, Report II.).

WELSH made it appear desirable to examine more closely the internal mechanism controlling 24-hour cycles in retinal pigment migration. Furthermore, he (1941) examined that the diurnal changes of the pseudopupil of the eye of *Cambarus bartoni* persisted for a period of several months even in constant darkness and he concluded that the migration of the retinal pigments was due to a hormone from the sinus gland whose innervation was traced by the vital staining method. Thus, it is advisable to study the phenomenon in a number of different species to determine which combinations of retinal pigments might be involved in such activities. The present investigation of diurnal change in the retinal pigments of some shrimps in our country is devoted to this purpose. Before going further, this author desires to express his thanks to Prof. Dr. S. NOMURA for his advice and criticism throughout the course of this work.

MATERIALS AND METHODS

The materials used in this investigation were obtained from fresh water pond in the vicinity of our laboratory and by dredging in the neighbouring sea of the Biological Station at Asamushi. The fresh water species was *Paratya compressa* (DE HAAN) and *Leander paucidens* (DE HAAN). The marine species was *Spirontocaris alcimede* DE MAN.

After a sufficient number of animals had been brought into the laboratory, the specimens were divided into two groups, one of which was placed in a white porcelain bowl under the illumination by a 40-watt electric lamp at a distance of 50 cm., while the second group was placed in a container covered by black paper in the dark-room of the laboratory. At least 48 hours were allowed for individuals to become adapted to light or to darkness.

After a period appropriate for adaptation, specimens were removed for fixation of the eye. According to KLEINHOLZ(1937), the term "daylight" eye (DL) is used to indicated the retina of a specimen that was kept constantly illuminated and which was fixed or examined in the daytime, while "night-light" eye (NL) represents the condition of the pigments in an illuminated retina that was fixed at night: conversely, "day-dark" eye (DD) and "night-dark" eye (ND) are employed to designate those specimens maintained in constant darkness whose retinal pigments were fixed during the day and at night, respectively. When a specimen was taken from the light adapted group, a similar specimen was removed from the container in the dark room for fixation at the same time.

At first, the animals were dropped into hot water (ca. 80°C) for about ten seconds to fix the positions of the retinal pigments in the eyes, and transferred to CARNOY's solution. Then they were retained in this fluid usually half a day.

Two methods of for histological study were used. In one method, the entire eye stalks were dehydrated by alcohol and then immersed into xylol to become transparent. The specimens treated in that way, were viewed by transmitted light under the low powers of the microscope and the positions of the distal retinal pigment were determined by using ocular micrometer. In the second method, when the exoskeleton had been sufficiently softened by the fixative, the eye stalks were excised and placed into soft paraffin. The tissues were allowed to become infiltrated with the wax for about three hours, with two changes of paraffin, and were then embedded. No difficulty was encountered in cutting serial sections at 10 μ. Some sections were subsequently stained with DELAFIELD's haematoxylin and eosin, and others were mounted unstained.

EXPERIMENTS

1. The diurnal rhythm of the distal pigment observed in the total preparation of eye stalks

Studying the clarified total preparation of eye-stalks of the shrimps fixed under the four experimental conditions mentioned above the position of the distal pigment was found as shown in Fig. 1.

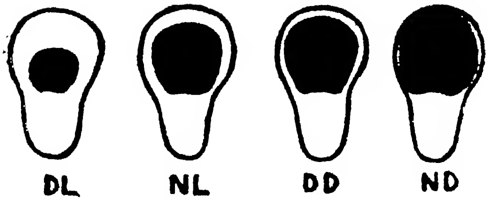

Fig. 1. Entire eye stalks of *Paratya compressa*, viewed by transmitted light through the low powers of the microscope and showing the position of the distal retinal pigments.

DL, from a day-light phase ; DD, from a day-dark phase ; NL, from a night-light phase ; ND, from a night-dark phase.

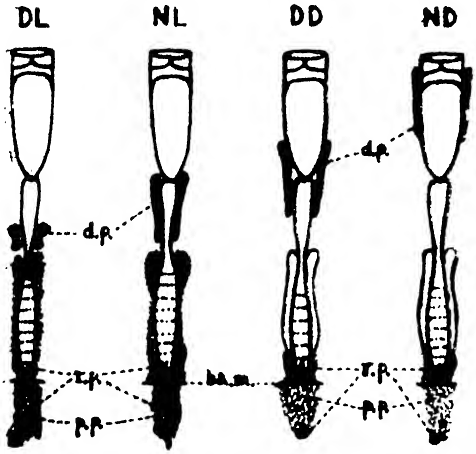

Fig. 2. The position of three kinds of the retinal pigments shows the diurnal rhythm of the pigments migration.

d. p.	distal pigment
p. p.	proximal pigment
r. p.	reflecting pigment
ba. m.	basement membrane

This physiological rhythm continued in animals kept in the dark at least for two weeks at temperature 20°~22°C.

2. The diurnal rhythm of the retinal pigments in the eyes.

Investigating by the sections of the eyes under the four experimental conditions, the position of three kinds of the retinal pigments shows the diurnal rhythm of the pigment migration (Fig. 2.)

The distal pigment displays diurnal periodicity, that is to say, it shows the day phase in the day time under the constant darkness and the night phase at night under the constant illumination. The reflecting pigment shows the night phase at night under the constant illumination. But the proximal pigment shows only the photomechanical change, the rhythm as shown by other two pigments was not recognizable. Three species selected for my expeiment had similar behavior concerning the retinal pigment, and gave the same, which has not been reported hitherto : —

Day-light eye

A. The distal pigment cells are to be found in the typical light-adapted state, the cells having moved proximally and come to rest against the proximal retinular cells.

B. The proximal pigment is also in the position typical for the light-adapted retina, some of it having migrated above the basement membrane to surround the rhabdome.

C. The reflecting pigment is also typically found above the basement membrane.

Night-light eye

A. The distal pigment, in spite of the fact that the retina has been under constant illumination, is situated at the distal ends of the cones.

B. The proximal pigment, however, is in the position in the usual light-adapted eye, as that of the day-light eyes.
C. The reflecting pigment is in the position characteristic of a light-adapted retina.

Day-dark eye
A. The distal pigment is in the position of the dark-adapted retina, forming a collar around the distal ends of the cones.
B. The proximal pigment has moved entirely below the basement membrane.
C. The reflecting pigment is in the position characteristic of a dark-adapted eye, most of parts above the basement membrane and remaining parts below the proximal pigment cells.

Night-dark eye
A. The distal pigment is in the position characteristic for a dark-adapted retina, forming a collar around the extreme distal ends of the cones.
B. The proximal pigment is also in the position in the dark-adaped eye, having migrated completely below the basement membrane.
C. The reflecting pigment is in the position typical for the dark-adapted eye.

3. The diurnal changes in the pseudopupil of the shrimp eye.

In superposition eyes such as *Astacus* and *Palaemon*, there is a well marked pseudopupil which at night gives origin to a general glow. This has been described by DAY (1911) in the crayfish *Cambarus*. This fact was reinvestigated exhaustively by WELSH (1939, 1941).

This "glow", which results from the reflection of light by the tapetal layer, covers a greater area of the eye in a completely dark-adapted animal than in one which is partly dark adapted. Hence the "glowing" area of the eye has sometimes been called a pseudopupil and this term was used in the present paper.

The present author examined the rhythm of the pseudopupil about two species of shrimps *Paratya compressa* and *Leander paucidens*. Preliminary observations on the shrimps demonstrated clearly that the diameter of the pseudopupil varied from day to night when animals were kept in the dark for periods of several days. It then became of interest to determine how they might be affected by temperature change.

Ten animals were placed in a black container on May 10th and kept in a dark room at 20°~22°C. Ten other shrimps were kept in a black vessel in the dark room on the same date and maintained at 8°C. Food was not given and water run ceaselessly. From this date until October, the test of two set of animals were made daily with few exceptions. Observations were made by removing a jar with its animal from a container and briefly illuminating the eye with a bright beam of light. The diameter of the pseudopupil was judged by looking at the eye along an axis which runs through the center of the eye and eye-stalk. Records were made in a graphic manner as shown in Fig. 3. The times for observation were varied from day to day, and only after several days a series of changes such as seen in Fig. 3. could be obtained.

In order to relate actual movements of the eye pigments to the diameter of the pseudopupil, other animals were killed in hot water and the eyes sectioned. It was found that the night condition, or larger pseudopupil, was seen only when the distal pigment was in the extreme distal position and all of the proximal pigment beneath the basement membrane, thus uncovering the tapetal or reflecting layer The decrease in diameter of the pseudopupil was found to be due to migration of the two screening pigments toward the positions normally occupied in the light.

Fig. 3. shows the changes observed in the pseudopupil in relation to time of day and temperature. It may be termed "average" results, for the figures are based on study of the records of all animals. In the first place it may be noted that there is a definite 24-hour cycle in the changes of the pseudopupil and in the second place that the "day phase" appears earlier and lasts longer at the lower temperature. And the rhythmic nature seen at the pseudopupil persisted for long periods of time in the shrimp, kept in constant darkness.

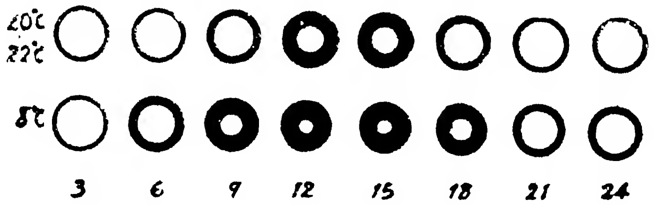

Fig. 3. Graphic representation of the 24-hour cycle in the pseudopupil of the shrimp maintained in constant darkness at 20°~22°C. and at 8°C. respectively.

4 The diurnal rhythm of the distal pigments in the shrimp eye.

In the case of the entire eyes of the shrimps, it has already been found that the distal pigment cells occupied their characteristic position when the animals were kept in constant darkness. Then, the analytical investigation of the rhythm of this pigment was required in datails. The observations of this purpose was made from May 20th to 22nd, 1942. The water temperature was 20°~22°C.

The results are shown graphically in Fig. 4. and Fig. 5. The points as plotted are average values of the measurements on the three animals at each time. Although there were individual variations, they were not great and all the animal responded essentially in group as a whole. The migration index used in the figures means the ratio of the distance from the outer layer of cornea to the extreme distal part of the distal pigment along the long axis of the eye stalk by the distance from the outer layer of cornea to the proximal boundary of the retinal pigment mass. It was possible to draw a curve which represents quite accurately the movements of the distal pigment cells. The outward movement of the pigment begins at about the time of sunset (at about $18^h\ 43^m$, May 20th) and it is slower than the inward movement occurring at the time of sunrise (at about $4^h\ 32^m$, May 20th).

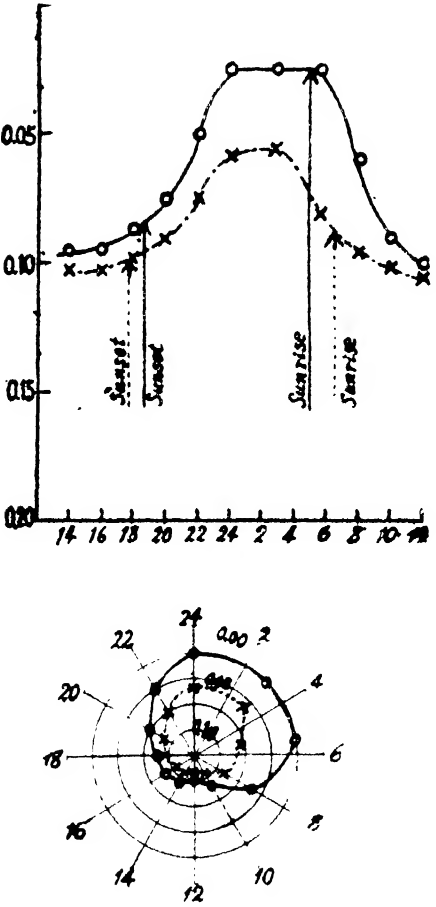

Fig. 4. and Fig. 5. The diurnal rhythmic curve of the movements of the distal pigment in the eyes kept in constant darkness

It is also to be noticed that the low temperature (8°C.) induced a low activity of the retinal pigments: at low temperature, the migration rate was smaller and the curve sloped more gradually than at high temperature. The temperature of 8°C. corresponded to the average temperature of March in Sendai and therefore the rise and decline of the curve is fitted to the time of sunrise (about $6^h\ 11^m$) and the time of sunset (about $17^h\ 36^m$). For those experiments just mentioned, only one species of *Paratya compressa* was employed.

5. The effect of low temperature upon the proximal pigment.

The analytical investigation by the section method for the eye, which was affected by the low temperature, showed that the proximal pigment among three retinal pigments migrated to or toward the positions characteristic of the light adapted eye, even though animals are kept in total darkness. This fact is very significant for the mechanism of the migration of the proximal pigment. (*cf.* Plate 1.)

DISCUSSION AND CONCLUSION

There will be much ignorance as to the nature of the mechanisms involved in the rhythmic activities of the animals. If the retinal pigments were uniform in their periodic responses under constant conditions, the problem as to the mediating agents would be relatively simple. But hitherto published accounts of the behavior of these pigments with the different crustaceans reveal perplexing complexities in this respect. Table 1. summarized the results obtained with various crustaceans for the purpose of the comparative consideration.

Table 1.

Crustacean		Retinal pigment			Investigator
Species	Family	Distal	Reflecting	Proximal	
Macrobrachium olfersii	Palaemonidae	NL	—	—	Welsh (1930)
M. acanthurus	Palaemonidae	NL	—	—	Welsh (1930)
Eusicyonia sp	Penaeidae	NL	Ab. or F.	—	Kleinholz (1937)
Trachypeneopsis mobilispinis	Penaeidae	NL	Ab. or F.	—	Kleinholz (1937)
Leander affinis	Palaemonidae	DD and NL	DD and NL	—	Welsh (1935)
Anchistioides antiguensis	Anchistiadae	DD and NL	DD and NL	—	Welsh (1935)
Leander paucidens	Palaemonidae	DD and NL	NL	—	Nagano (This paper)
Paratya compressa	Atyidae	DD and NL	NL		
Leander tenuicornis	Palaemonidae	—	NL	— —	Nagano (This paper) Welsh (1935)
Latreutes fucorum	Hippolytidae	—	NL	NL	Welsh (1935)
Hippolyte varians	Hippolytidae	—	—	—	Kleinholz (1938)
H. pleuracantha	Hippolytidae	—	DD	DD	Kleinholz (1938)
Cambarus virilis	Potamobiidae		—	DD	Bennitt (1932)
Peneopsis goodei	Penaeida	F.	F.	DD	Welsh (1935)
Portunus anceps	Portunidae	DD	Ab.	DD	Kleinholz (1937)
P. depressifrons	Portunidae	DD	Ab	DD	Kleinholz (1937)
Parthenope serrata	Parthenopidae,	DD	Ab.	DD	Kleinholz (1937)
Calappa flammea	Calappadae	DD	Ab.	DD	Kleinholz (1937)

——....pigment shows normal photomechanical changes but shows no periodicity.
Ab. ....pigment is absent from the retina.
F. ....pigment is present, but undergoes no positional changes.
NL ....rhythm in a night-light eye, the pigment moving to a typical dark position.
DD ....rhythm in a day-dark eye, the pigment moving to the position characteristic of a light-adapted retina.

In the first four species of macrurans, there is a uniformity of response in that the same set of retinal pigments, the distal, shows persistent periodic movements in the same direction under the same conditions of illumination. I should like to call those group Type I. The next two species of shrimps are unusual in that they show evidence of possessing a double rhythm; not only are distal and reflecting retinal pigments involved in the periodic migration, but the periodicity occurs twice within a 24-hour cycle, once during the daytime, when the animals are maintained in darkness, and once at night when the shrimps are kept under constant illumination. I should like to call those animals Type II.

The two shrimps of our experimental objects showed also a different type, as follows. That is to say, the distal pigment has the periodicity of migration twice within a 24-hour cycle, the reflecting pigment shows only once at night when the shrimps are kept under constant illumination. Then I should like to call those shrimps Type III.

Now, in *Leander tenuicornis* and in *Latreutes fucorum*, the situation is reversed, the reflecting pigments showing the rhythm, while the distal pigment cells respond only to differences in light intensity. Hence, I should like to call this group of these animals Type IV.

If endocrine control of these two pigments, distal and reflecting, is universally present in the crustaceans, such differences in response may possibly be due to threshold variations in reactivity to the same hormone, or, there may be two hormones involved, one for the distal pigment and one for the reflecting pigment. At any rate, it may be said that Type III is the intermediate of transforming group between Type II and Type IV.

The mechanism involved in the migration of the retinal pigments in the four brachyurans (*Portunus anceps*, *P. depressifrons*, *Parthenope serrata* and *Calappa flammea*) reported by KLEINHOLZ is open to analysis chiefly because so little is known about them (Type V.). *Cambarus* and *Peneopsis* seem to fall into the same group (Type VI), in that the proximal pigment shows the rhythm.

The mediating agency for the migration of the proximal pigment is unknown. In *Palaemonetes* (KLEINHOLZ, 1936) and *Paratya* (NAGANO, 1947), the eye stalk extracts which affect the distal and reflecting pigments have no effect on the behavior of the proximal pigment. Early workers on the migration of the proximal retinal pigment were of the view that this activity was under nervous control. But, as BENNITT (1932, b) states, the main argument against this belief is that no efferent nerve fibers have been found supplying these cells, their only nervous connection apparently being afferent fibers going to the optic ganglia (PARKER, 1895). BENNITT (1932, b) said that an endocrine control may be involved. Recently, WELSH (1941) pointed out the innervation of the sinus gland whose function is considered to supply the eye stalk hormone. That is to say, motor fibers from one of the oculomotor nerves go to the region of the sinus gland, and fibers from the medulla terminalis (optic ganglion IV) and from the supraoesophageal ganglion (brain) join to form the sinus gland nerve, which can be traced into the tissue of the gland. It may be suggested that tonic inhibitory centers in the medulla terminalis or supraoesophageal ganglion normally prevent the release of the retinal pigment controlling hormone. Stimulation of the eye by light reduces or abolishes the activity of these inhibitory centers allowing the release of the hormone. The evidence for this is largely indirect and based on the observed effects of chloretone anaesthesia, (WELSH, 1930; WELSH, 1941), O_2-deficiency (BENNITT and MERRICK, 1932), low temperature (CONGDON, 1907, WELSH, 1941, NAGANO, in this paper) and general inactivity (the day phase or period of "sleep", WELSH, 1941) These factors, which would tend to lower the activity of nervous centers, cause a migration of retinal pigments to or toward the positions characteristic of the light, even though animals are kept in total darkness.

The regularly recurring 24 hour cycles in pigment migration in the eye of the shrimps under constant external conditions might, therefore, be due to regular variation in the activity of nervous inhibitory centers, causing the variation in the amount of hormone released from the eye stalk (probably from the sinus gland).

SUMMARY

1. Persistence of a diurnal rhythm in the migration of the retinal pigments of three species of shrimps, *Paratya compressa* (DE HAAN), *Leander paucidens* (DE HAAN) and *Spirontocaris alcimede* DE MAN in constant illumination or darkness, is reported.

2. The diurnal rhythm of the distal pigment in the eyes of those shrimps was observed in sections and total preparations of the eye.

3. The distal pigment of the two species of the fresh water shrimps has the periodicity of migration twice within a 24-hour cycle, the reflecting pigment shows only once the periodicity of migration at night when the shrimps are kept under constant illumination.

4. The diurnal rhythm of the pseudopupil can be seen in the three species.

5. It may be noted that the "day phase" of the pseudopupil appears earlier and lasts longer at the low temperature.

6. The curve representing the daily movements of the distal pigment cells is obtained by using the migration index of its pigment.

7. The proximal migration was induced by low temperature to assume the position characteristic of the light adaptation, even though animals were kept in darkness.

8. It may be said, that the migration type of the eye pigment may be divided into six types, and the Type III found in my experiment is the intermediate form between Type II and Type IV.

9. The persistence of the diurnal rhythm suggests that an internal rhythmical mechanism may be responsible, in part, for pigment movements in crustacean eyes.

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PHYSIOLOGICAL STUDIES ON THE PIGMENTARY SYSTEM OF CRUSTACEA.

V. DRUG ACTION UPON THE PIGMENTARY SYSTEM OF A SHRIMP.*)

BY

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(With 1 Text-figure)

(Received August 31, 1949)

INTRODUCTION

While the effect of drugs on vertebrate melanophores has been determined, few similar investigations have been made on crustacean chromatophores. ABRAMOWITZ (1936) succeeded, however, in expanding the chromatophores in the crab *Uca* with intermedin. The same result was also obtained by BÖTTGER (1935) in the shrimp *Crangon*. But since the reactions of the chromatophore are reversed in crabs and shrimp, and in another shrimp such as *Leander adspersus*, HANSTRÖM (1937) was not able to find any expansion of the chromatophores under the influence of intermedin. Then BÖTTGER's result ought to be reinvestigated.

There exists some evidence for an agreement in action and properties between intermedin and the pigmentary hormone in Decapods, but of course there are some important differences.

Now in the frog, intermedin and adrenaline act antagonistically, the former substance expanding, the latter contracting the melanophores. It is true that KALMUS (1938) did not observe any action of adrenaline on the chromatophores

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*) This report is a part of the series of papers submitted to the Faculty of Science, Tôhoku University, in partial fulfilment of requirements of the degree of Doctor of Science, March 1948.

of *Astacus fluviatilis* whose chromatophore reactions belong to the same type as those in *Leander adspersus*. On the other hand, BEAUVALLET and VEIL (1934) stated that they have been able to expand the chromatophores in *Palaemon* (*Leander*) *squilla* with adrenaline, and to contract them repeatedly with eye stalk extracts and again expand them with adreanline in this same shrimp. Thus the eye stalk extracts of this animal acts in this instance antagonistically to adrenaline, which fact lends some support to the physiological comparison between the common crustacean eye stalk hormone and intermedin. ABRAMOWITZ, A.A. and R.K. ABRAMOWITZ (1938) have tested the effect of sixteen different, chemically pure drugs upon both the normal and blinded crabs. The results were ineffective in causing melanophore expansion of the crabs.

As the facts mentioned above are conflicting, the present author has undertaken a reinvestigation of the subject. The work was carried out at the Biological Institute in Sendai at 1943. It was accomplished under the direction of Prof. Dr. S. NOMURA to whom I am deeply grateful for kind guidances and suggestions.

MATERIAL AND METHOD

By reason of its ready observation and the ease with which it can be collected and cared for in the laboratory, *Paratya compressa* (DE HAAN) was used as the material of present investigation.

Twelve different, chemical drugs were injected into both normal and blinded animals. The water solution of those drugs of various concentrations was introduced into each of ten animals. A dose of 0.025 cc. of each drug was injected into the haemocoecal cavity of the shrimp from the lateral side of the third abdominal segment.

Not only the occurrence of the change in the dermal chromatophore, but the behavior of the eye pigments were also investigated by the method of the section of the eye stalk after the lapse of 15 minutes of injection. The fixing and staining method of the eye section should be referred to the previous report (Report II, 1947).

RESULTS

The action of twelve drugs upon the dermal chromatophore of the shrimp was summarised in the following Table I.

Table I

Effects of twelve drugs on normal and blinded shrimps.

Drug	Concentration	Blinded (dark animals)		Normal (light animals)	
		General effect	Chromatophorotropic activity (chromatophore contraction)	General effect	Chromatophorotropic activity (chromatophore expansion)
Atropine sulphate	10^{-4}	-	-	- -	- -
Morphine sulphate	〃	-	-	- -	- -
Pilocarpine hydrochloride	〃	- -	- -	- -	-
Strychnine sulphate	〃	paralysis	slight effect(+)	paralysis	- -
Veratrine sulphate	〃	death	- -	death	slight effect(+)
Caffein	〃	- -	- -	- -	——
Nicotine	〃	prostration	slight effect(+)	prostration	- -
Insulin Toronto	〃	death	-	death	- -
Hinterin*	〃	-		-	+++
Hypophorin**	〃	-	-	——	+++
Adrenaline	10^{-3}	death	-	death	slight effect(+)
	10^{-4}	paralysis	slight effect(+)	paralysis	- -
	10^{-5}	- -	- -	- -	- -
Acetylcholine	10^{-4}	death	- -	paralysis	——
	10^{-5}	paralysis	slight effect(+)	- -	slight effect(+)
	10^{-6}	- -	-	- -	- -

- - ...... No effect +++ ...... considerable effect

* Hinterin ...... Medicament of anterior lobe of pituitary. Sankyo Co.

** Hypophorin ...... Medicament of posterior lobe of pituitary. Sankyo Co.

None of these drugs with the exception of hypophyseal hormone (Hinterin and Hypophorin) produced definite, positive results in normal light specimens, and all had no effect upon the chromatophores in blinded dark specimens. These drugs have, therefore, no direct or indirect action on contracted or expanded (with two exceptions) chromatophores. **We did** not repeat the injection in normal animals during the night (nocturnal or pale phase in absolute darkness), although possibly some of these drugs may effect a release of the hormone from the chromatophorotropic gland such as sinus gland.

Neither these drugs had effect upon the movement of the pigmentary system in the eye, with two exceptions. The two exceptions were in the case of adrenaline and acetylcholine. These drugs have affected the movement of the proximal pigment cells, so that the pigments migrated proximally in the night dark eye (Fig. 1). Then they may be concluded as the nervous stimulant as if the light affected the proximal pigment.

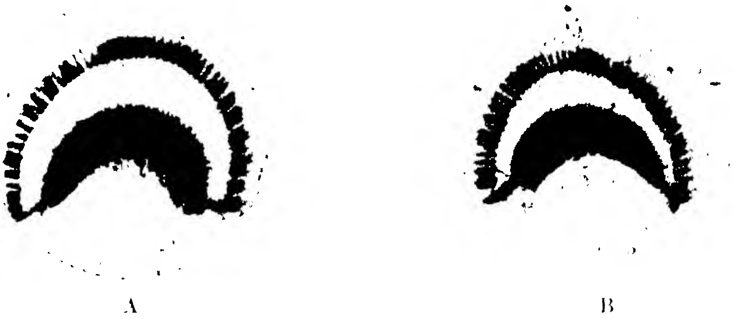

Fig. 1. The position of migrated proximal pigment by injection of adrenaline (A) and acetylcholine (B) in the dark adapted eye.

DISCUSSION AND CONCLUSION

Adrenaline, practically universally, produces melanophore contraction in vertebrates in extremely small doses. ABRAMOWITZ and ABRAMOWITZ (1938) have stated that various drugs had no effect on the chromatophore in the fiddler crab, *Uca pugilator*. Strong doses produced usually death in animals. Only slight activity of adrenaline and acethylcholine was recognized in this experiment as regards the dermal chromatophore.

But the hypophyseal hormone obtained from anterior or posterior lobes of vertebrates (Hinterin and Hypophorin) induced the remarkable expansion of the dermal chromatophore in this shrimp. This result may be also explained by the fact that the expansion of the chromatophore is caused by unfavorable condition of the animal.

It has already been confirmed that the crustacean pigmentary hormone affects the chromatophores in vertebrates (KOLLER and MEYER, 1930; KROPP and PERKINS, 1933; NAGANO, 1943), and that the intermedin of vertebrates induces the expansion of the readily contracted chromatophore of *Uca* (ABRAMOWITZ, 1936). But it must be noticed that any drug in my experiment can not induce the contraction of the chromatophore. That is to say, there is no drug that affects the chromatophore as the extract of the eye stalk.

But, as the responding tissue in two different animals, vertebrates and crustaceans, are physiologically and anatomically different, it is not surprising that they react in different ways to the same substance.

I shall pass to another subject. The mechanism involved in the control of retinal pigment migration in crustaceans is not yet completely known. Recent studies on *Palaemonetes vulgaris* by KLEINHOLZ (1936) present evidence for a humoral control of distal and reflecting pigment in the retina, but proximal pigment appears not to be affected by the same eye stalk extracts which activate the first two sets of pigments. In this point, my previous works (Report II, IV, 1943, 1948) have dealt with the matter in details. Then it will draw out attention that the proximal pigment in the shrimp eye was affected only by adrenaline and acetylcholine in this experiment. That is to say, it is possible to conclude that the proximal pigment is under nervous control and other two retinal pigments are under hormonal control.

SUMMARY

1. Twelve different drugs were ineffective in causing the contraction of the dermal chromatophore in *Paratya compressa* (DE HAAN).

2. The vertebrate hypophyseal hormone (Hinterin and Hypophorin) induced considerable expansion of the chromatophore of this shrimp.

3. The proximal pigment cells in the eye seems to be under nervous control by reason of its migration by adrenaline and acetylcholine; distal and reflecting pigment cells seem to be under hormonal control.

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STUDIES ON MARINE NON-COLORED FLAGELLATES, *MONAS* SP., FAVORITE FOOD OF LARVAE OF VARIOUS MARINE ANIMALS.

I. PRELIMINARY RESEARCH ON CULTURAL REQUIREMENTS.

BY

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INTRODUCTION

It has been shown that the larvae of oyster and many other bivalves and also echinoderms can satisfactorily be reared with a culture of non-colored flagellate, presumably identified as *Monas* sp. (IMAI, T. and M. HATANAKA, 1949). This type of diet, as compared with colored algal diet, has advantages of 1) the readiness for preparing any amount of culture in short time and of 2) the easiness of keeping the culture medium of larvae in favorable condition. Such advantages have brought the success of rearing larvae in small vessels without much trouble of changing sea water during culture. Futhermore this new principle of culture has been proved applicable to large scale propagation of oyster larvae in tanks (IMAI, T. and M. HATANAKA, 1944).

During the spawning season of 1941, we succeeded in isolating the best fitted flagellate from the sea water of Mangoku-ura, a natural seed-oyster farm near our laboratory. We have chiefly used this strain, *Monas* sp. No. 34, for the culture experiments ever since. The culture method of *Monas* sp. applied to the larvae rearing experiment in last report was as follows: MIQUEL's sea water was prepared and 2% of hay infusion was added. Then a few cc. of stock culture of *Monas* were inoculated. The culture flasks were kept in thermostat at 20°C. There occurred a rapid growth of bacteria and then followed a rapid growth of *Monas*. In this culture medium *Monas* density often reached nearly half a million per cc. in a few days. So far, no critical examination was made on the nature of *Monas* growth and also on the nutritional requirements in culture medium.

Series of experiments were made with the purpose of catching on the mechanism of *Monas* growth and of improving the culture method. Their results are to be given in this paper. Final aim of this research was to find out and settle the culture method which gave uniform high density of *Monas* in a medium harmless for larval growth.

Ordinary fixatives could not be used for handling *Monas* as it was ready to collapse. In order to count the density of its population, LUGOL-eosin solution was satisfactory enough to use. *Monas* was readily stained and kept its shape properly for over 48 hours if treated with 5~10% of LUGOL-eosin solution. But with a lower concentration of it, we could make daily counting by the help of THOMA's haemocytometer under the microscope.

Occurrence of this kind of flagellates in natural sea water was rather astonishing. We counted from a few hundreds to several thousands of *Monas* in coastal waters. They were particularly abundant in brackish water and often reached a density as high as one tenth million per cc. of sea water. Significance of occurrence of protozoan fauna in sea water was suggested by LACKEY (1936). Our ecological survey of natural seed-oyster farm of Mangoku-ura confirmed the important rôle played by *Monas* group in marine production (IMAI, T., M. HATANAKA and R. SATO, unpublished). This problem will be discussed in some other place.

EXPERIMENTS.

1. *Use of Glucose as Organic Nutrient.*

As a culture medium of *Monas*, MIQUEL's sea water with 2% of hay infusion was used (IMAI, T. and M. HATANAKA, 1949). Extract of eel-grass, *Zostera marina*, and soil extract were also tried in place of hay infusion with various degrees of success. In our study on culture medium, it was necessary to begin with finding out what is the essential element in hay infusion or in organic substances added. Main constituents of these plant organic matters which had direct influence on culture of *Monas* should be carbohydrates and proteins. In our experiences of larvae rearing, the culture medium with organic substances rich in protein content, particularly of animal origin, always gave negative results, possibly due to the harmful effects of decomposition products of organic nitrogen.

Therefore, it was hoped to keep out of adding organic nitrogen in culture medium. Naturally our first effort was to find out whether we could substitute hay infusion with carbohydrate such as glucose.

Sea water was taken from the surface of Onagawa Bay, in front of the laboratory and was Miquel-ized according to the direction modified by ALLEN and NELSON (1910). Various amounts of glucose were added in 500 cc. flasks filled with the prepared sea water. Then a few drops of stock culture of *Monas* were inoculated. Culture flasks were kept in dark thermostat with constant temperature at 25°C. They were aerated by frequent shaking. The results of the experiments are shown in Table I.

TABLE I.

Growth of *Monas* in MIQUEL's sea water enriched with different amounts of glucose. Incubation at 25°C.

Glucose added.	Maximum Population of *Monas* per cc.	Period of Incubation in Days.
0.5 %	20,000	5
0.1 %	10,000	5
0.01 %	250,000	5
— —	Less than 1,000	5

As is clear from Table I, *Monas* grew well in MIQUEL's sea water enriched with 0.01% of glucose. Addition of more glucose rather had ill effects on culture. One reason for that seemed to be due to the oxygen defficiency as was supposed from the results shown by WAKSMAN *et al.* (1935).

Beside glucose, cane sugar, maltose, and starch brought good results when they were added in quantity with equivalent carbon content. Addition of alcohols and pentose also gave positive result but the maximum population was much smaller than those mentioned above. Thus it has been proved that we can make a good culture of *Monas* in solutions enriched with known chemicals only and that we can use carbohydrate as sole organic nutrient.

In order to compare the growth of *Monas* in solution with hay extract and that with glucose, the following experiment was made. MIQUEL's sea water was enriched with 2% hay infusion and 0.01% of cane sugar respectively. They were inoculated with a few drops of stock *Monas* culture and were incubated at 15°C. and 25°C. respectively. Results of *Monas* growth are shown in Table II.

TABLE II.

Comparison of growth of *Monas* in culture media enriched with hay infusion and cane sugar.

Organic Enrichment.	Temperature.	Maximum Population of *Monas* per cc.	Period of Incubation in Days.
0.01% cane sugar	15°C.	1,230,000	5
2% hay infusion	15°C.	440,000	5
0.01% cane sugar	25°C.	860,000	3
2% hay infusion	25°C.	380,000	3

Culture medium enriched with 0.01 % cane sugar showed much better growth of ***Monas*** than that with 2 % hay infusion. It was already known (IMAI T and M. HATANAKA, 1949) that by adding more hay infusion we could have higher density of *Monas* up to one million per cc. But such high concentration of hay infusion had rather harmful effect on living larvae. Therefore, cane sugar is considered much better as organic enrichment than hay infusion.

2. *Requirements of Inorganic Nutritive Salts.*

In the foregoing experiments MIQUEL's sea water was used thoroughly. Our second concern was to prove the necessity of adding MIQUEL solution and further to follow out the esssential inorganic salts required for the culture of *Monas.* The results of experiments in Table III show clearly the necessity of adding MIQUEL solution to culture medium. HERBST's artificial sea water was prepared, and the growth of *Monas* population was followed in the media with or without MIQUEL solution. For the preparation of HERBST's artificial sea water, 26.3 g. of sodium chloride, 0.7 g. of potassium chloride, 11.93 g. of magnesium sulphate, 1.508 g. of calcium chloride and 0.45 g of sodium bicarbonate were added to 1 litre of distilled water.

TABLE III.

Growth of *Monas* in artificial sea water with or without enrichment of MIQUEL solution, at 25°C.

Organic Nutrients.	Inorganic Nutrients	Maximum Population of *Monas* per cc.	Period of Incubation in Days
1 % hay infusion	——	140,000	4
1 % soil extract	——	75,000	3
2 % soil extract	——	170,000	3
0.01 % cane sugar	——	80,000	4
0.01 % cane sugar	MIQUEL solution	722,000	5

It is clear from the Table that *Monas* grew in the artificial sea water enriched only with organic nutrients but the maximum population was not comparable with the culture in which both of organic nutrients and MIQUEL solution were added. It may be concluded then that besides organic nutrients a minute quantity of inorganic nutrients is essential for the growth of *Monas*.

MIQUEL's sea water was originally formulated for the purpose of diatom culture. After knowing its necessity as inorganic nutrients, it was asked how essential each inorganic salt was for *Monas* culture. In order to make this point clear, another set of experiment was carried out. Kind and amount of inorganic salts added are shown in Table IV with the results of population growth of *Monas*. As an organic nutrient, 0.01% of glucose was added throughout. They were incubated at 25°C.

TABLE IV.

Growth of *Monas* in natural sea water enriched with 0.01% of glucose and different combinations of inorganic salts, contained in MIQUEL solution. Incubation at 25°C.

Inorganic Salts added.	Maximum Population of *Monas* per cc.	Period of Incubation in Days.
0.04 % potassium nitrate.	40,000	4
0.005 % sodium phosphate.	10,000	4
0.04 % potassium nitrate & 0.005 % sodium phosphate.	310,000	4
0.04 % potassium nitrate, 0.005 % sodium phosphate & 0.005 % calcium chloride.	710,000	4
0.04 % potassium nitrate, 0.005 % sodium phosphate, 0.005 % calcium chloride & 0.0075 % ferric chloride.	290,000	4
Standard Amount of MIQUEL Solution.	440,000	4

Both potassium nitrate and sodium phosphate, if added separately, could not give so much effect on the growth of *Monas*. But when added together, they brought about good results. Addition of calcium chloride was likely to improve the culture medium, but ferric chloride showed no beneficial effect on it so far. The result of this experiment indicates that at least nitrate, phosphate and calcium salts are essential as additional inorganic nutrients for the culture of *Monas*.

It was expected that nitrate, as nitrogen source, could be substituted by ammonium salt, and it was proved clearly by following experiment. Sea water was enriched with 0.01 % cane sugar.

TABLE V.

Growth of *Monas* in sea water enriched with 0.01 % cane sugar and ammonium salts. Incubation at 25°C.

Inorganic Salts added.	Maximum Population of *Monas* per cc.	Period of Incubation in Days
0.01 % ammonium chloride & 0.005 % sodium phosphate	645,000	4
0.015 % ammonium phosphate	520,000	4

It is understood from the result that inorganic nitrogen can be supplied in either form of nitrate or ammonium salts. It was further shown that ammonium phosphate could be used as nitrogen and phosphorus sources.

So far the requirements of inorganic nutrients in *Monas* culture were analysed and it was proved that **MIQUEL** solution is a fairly good combination of inorganic nutrients essential for the growth of *Monas*. It was still left unsolved that what would be the most suitable concentration of these inorganic salts for the optimum growth of *Monas*. In order to solve this problem following experiment was planned. Sea water was diluted with twice as much volume of fresh water to give optimum salinity for culture as will be referred to later. It was then enriched with different amounts of MIQUEL solution and 0.01 % cane sugar. Inoculated with a few drops of stock culture, it was incubated at 25°C.

TABLE VI.

Effects of concentration of inorganic nutrients on the growth of *Monas*, in 30 % sea water with 0.01 % of cane sugar. Incubation at 25°C. Concentration of inorganic nutrients are expressed in unit of MIQUEL solution added for standard MIQUEL's sea water.

Amount of MIQUEL Solution.	Maximum Population of *Monas* per cc.	Period of Incubation in Days.
——	20,000	2
1/200 of standard unit	440,000	4
1/40 ″ ″ ″	770,000	4
1/20 ″ ″ ″	770,000	4
1/10 ″ ″ ″	580,000	4
1/4 ″ ″ ″	740,000	4
1/2 ″ ″ ″	630,000	4
1 ″ ″ ″	610,000	4

It can be said from the result obtained that we can diminish the amount of inorganic nutrients as low as to 2.5 % of the standard MIQUEL's sea water without any indication of inorganic deficiency.

3. *Optimum Salinity of Culture Medium.*

In order to find the optimum salinity for *Monas* culture, culture media with different salinity were prepared by mixing the concentrated sea water, evaporated at low temperature, natural sea water and fresh water. They were enriched with 0.01 % cane sugar and 1/40 unit of MIQUEL solution, inoculated with a few drops of stock culture of *Monas* and incubated at 25°C.

TABLE VII.

Effect of salinity on *Monas* growth. Enriched with 0.01 % of cane sugar and 1/40 unit of MIQUEL solution. Incubation at 25°C.

Percent of Sea Water.	Chlorinity, Cl. ‰.	Maximum Population of *Monas* per cc.
10	1.81	330,000
30	5.40	640,000
50	9.05	470,000
80	14.48	270,000
100	18.13	220,000
110	20.00	340,000
140	25.00	370,000

The result of experiment shows that *Monas* can grow well in wide range of salinity condition. However, the optimum salinity for its growth seems to be in the diluted sea water between 30–50 per cent natural sea water.

4. *Optimum Temperature Condition for Monas Culture.*

MIQUEL's sea water enriched with 0.01 % of cane sugar was inoculated with a few drops of *Monas* culture and was incubated under various temperature conditions

TABLE VIII.

Growth of *Monas* under different temperature conditions, in MIQUEL's sea water enriched with 0.01 % of cane sugar.

Temperature °C.	Maximum population of *Monas* per cc.	Period of Incubation in Days.
30 (29–32)	50,000	6
25 (24–26)	860,000	4
20 (19–21)	1,080,000	7
15 (14–17)	1,230,000	6
10 (8–13)	127,000	7
5 (2– 6)	20,000	11

Growth of *Monas* went on well at temperatures between 10–25°C. of which the best was at 15–20°C. As for heat resistance, it was found that *Monas* was killed when exposed to temperature as high as 40°C. for ten minutes.

5. *Mode of Monas Growth, with Reference to Its Relation to Bacterial Growth.*

We have described the results of the experiments carried out for improving the method of *Monas* culture. Now we would like to see how the *Monas* grows in the culture medium. A typical example of its population growth is summarized in Table IX.

TABLE IX.

Population growth of *Monas* in MIQUEL's sea water enriched with 0.01 % of cane sugar. Incubation at 15°C.

Period of Incubation in Days	Number of *Monas* in 1,000 per cc.	pH of Culture Medium.
0	1.5	8.15
2	20	8.05
3	90	8.05
4	870	8.10
5	1,230	8.10
6	820	8.10
7	1,174	8.20
9	880	8.30

In first few days of incubation there occurred a rapid growth of bacteria, which was succeeded by a rapid growth of *Monas* soon afterward Population peak was reached on the 5th day and lasted for a few days. As bacterial decomposition of organic substance proceeded, pH of culture medium decreased but soon recovered again.

Monas is supposed to be a holozoic protozoa so that its development is necessarily supported by existence of bacterial population. In another experiment we fed *Monas* with bacteria which had been cooked by heat and washed in sterilized sea water, but no difference was observed in the mode of its growth. Such evidence seems to indicate that though *Monas* might have also a saprophytic mode of nutrition, such mode is by no means as important as holozoic one.

Body size of *Monas* showed changes in the course of culture. In early period of incubation, *Monas* grew up by ingesting a large amount of bacteria and reached beyond 10 μ in length, but as the population increased by binary fission it began to reduce its size until it got to the length as small as 2 μ after the population peak had passed.

In the foregoing experiment, no critical consideration had ever been made as to the type of bacteria grown in the culture. As *Monas* feeds on bacteria it should be worthwhile to see if this flagellate has any preference on the kind of bacteria or not. Test was made as is shown in Table X. Eleven strains of known forms of bacteria and nine unidentified strains, isolated from sea water, had been cultured on bouillon agar slant. Dense suspension of bacteria was prepared by putting 5 of 4 mm. loopful bacteria in 50 cc. of 30 % sea water, and then *Monas* was inoculated.

TABLE X.

Growth of *Monas* in dense suspension of single strain of various bacteria. Incubation at 25°C.

Bacteria.	Maximum Population of *Monas* in 1,000 per cc.
Bacillus subtilis	3,520
Bacterium fluorescens	2,500
Bacterium coli No. 1	4,780
〃 〃 No. 2	3,130
〃 〃 No. 3	3,080
Bacterium proteus 52	4,120
〃 〃 80	4,450
〃 〃 81	3,330
〃 〃 99	4,570
〃 〃 101	3,230
Bacterium prodigiosum	3,860
Bacteria isolated from sea water No. 1	1,750
〃 〃 No. 2	2,640
〃 〃 No. 3	5,220
〃 〃 No. 4	3,360
〃 〃 No. 5	3,610
〃 〃 No. 6	5,010
〃 〃 No. 7	3,390
〃 〃 No. 8	1,130
〃 〃 No. 9	2,090

Though there was observed slight variations in maximum population of *Monas*, it can be said that all of these bacteria are suitable for *Monas* culture. Therefore, we are of the opinion that we can depend on any common bacteria which multiply well in our culture media for *Monas* culture. It was also proved in this experiment that we could get higher density of *Monas* with higher density of bacteria. For the purpose of rearing larvae the use of such dense suspension of bacteria is suggested as ideal if it is not much troublesome.

DISCUSSION AND CONCLUSION

In this study, we made an analysis on the cultural requirements of marine flagellate, *Monas* sp., which was the favorite food organism for larvae of lamellibranchs and echinoderms. As a culture medium of it, we originally used the MIQUEL's sea water enriched with 2% of hay infusion. Therefore we took up this medium as a start for analysis of the essential elements necessary for the growth of *Monas*. Our final aim was to find the optimum conditions for *Monas* culture which is also favorable for feeding larvae.

From the results obtained, we may conclude that good culture of *Monas* could be obtained in sea water when small amount of glucose or any other carbohydrate was added as a sole organic compound. But at such addition of carbohydrate, enrichment of inorganic nitrogen in the form of nitrate or ammonia as well as phosphorus in such form as sodium phosphate was also required. Addition of calcium chloride was found effective in promoting the growth of *Monas*. Beneficial effect of ferric chloride was not confirmed yet. Optimum condition for *Monas* culture can be summarized as follows·

Salinity	5-10 in Cl.‰: about 30-50% sea water.
Glucose	About 100 mgm/L.
Potassium nitrate	40-400 mgm/L.
Sodium phosphate	5-50 mgm/L.
Calcium chloride	5-50 mgm/L.
Temperature of Incubation	15-20°C.

Using this culture medium we could obtain a uniformly dense population of *Monas* often as high as over a million per cc. As to certain irregularities in *Monas* growth observed among the results of various experiments, we are of the opinion that they were possibly due to the difference in quality and quantity of organic matter in sea water, sampled on different occasions.

During incubation there occurred a rapid development of bacteria, then followed the development of *Monas*. As *Monas* fed on bacteria, its density had intimate relation with the density of bacterial population as supposed from the results shown in Table X. Accordingly, it can readily be understood that the cultural requirement of *Monas* is in turn in close connection with that of bacterial development. As a matter of fact, the culture medium which we confirmed to be favorable, has the similar constituents to the medium frequently used for the culture of marine bacteria. Bacterial action of decomposing organic matter in sea water on adding glucose has thoroughly been studied. We can cite the works of WAKSMAN (1935), ZOBELL (1941) and many others. In the present study no critical examination was made as to the bacterial activity itself. But from the bacteriological studies just referred to and also from our own observations, mechanism of *Monas* growth can be explained. In the culture, bacteria develop very rapidly taking glucose as energy source of activity and carbon source for building cells. Both nitrate and phosphate are then used as nitrogen and phosphorus sources for building cells. It is out of question that the organic matter and the inorganic nutrients contained in natural sea water also take part in this process of organic decomposition and becterial synthesis. The bacteria thus multiplied, in turn, are ingested by *Monas*, and give rise to the growth of its population.

Knowing that *Monas* feeds on bacteria, there occurs an important question as to the comparable food value of bacteria and *Monas* as diet of larvae of marine animals. That is the question whether the food value of bacteria is improved by being transferred to *Monas* or not. We may present here some facts introducing a light on this problem. In feeding experiment we found that bacteria themselves were not a suitable food for larvae. As foods are collected by current caused by ciliary movement, no selection of bacteria or *Monas* can be considered. Examining under the microscope, we noticed plenty of small particles, presumably bacterial cells, rotating inside of larval stomach, but, strange to say, no flagellate in good shape was found there. It was due to the fact that *Monas* collapsed soon after they had been taken in through the gut. This can be demonstrated beautifully by feeding larvae with *Monas* stained by neutral red. Such evidence seems to indicate that transformation of bacteria into *Monas* increases the readiness in digestion for larvae if there is no addition in its chemical food value. Similar evidence was also noticed in the culture experiments of water fleas, *Moina macrocopa*, on bacterial and flagellate diets (IMAI, T. and R. SATO, 1949). On adding flagellates to bacterial diet, *Moina* increased the rate of development, production of youngs and duration of life span.

Final aim of the study on *Monas* culture is to obtain uniform high density of food organism in the culture medium which has no ill effect on larval life. Culture condition, we reached in this study, seems to be fairly satisfactory for larvae rearing. But there remains many problems left for future studies.

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STUDIES ON PHYSIOLOGY AND ECOLOGY OF PLANKTON.

III. CHANGES IN RESPIRATORY QUOTIENT DURING EMBRYONIC DEVELOPMENT OF A DAPHNID, *SIMOCEPHALUS VETULUS* (O. F. MÜLLER).

BY

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(With one Text-figure)

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INTRODUCTION

Judging from the value of the respiratory quotient, NEEDHAM (1931, '32, '33 & '42) holds a view that carbohydrate metabolism is intimately associated with earlier developmental stages in various kinds of animals. He asserts also that in later stages protein and fat are utilized successively as energy sources of development of animals. Recently, on the other hand, ÖHMAN (1940, '45) studied the respiratory metabolism of sea-urchin eggs, and found that fat was consumed during the earlier stage in development.

The summer egg of the daphnid is centrolecithal and the segmentation is superficial. As the development proceeds, the egg yolk is absorbed gradually but more rapidly on the ventral side than on the dorsal one. In newly released young, the yolk is absorbed almost entirely, and only a small amount remains on the dorsal side of the body. Changes in the value of respiratory quotient of embryo may, therefore, be expected with consumption of the yolk material. The present study deals with the changes in respiratory quotient during the embryonic development of *Simocephalus vetulus*.

The author wishes here to express his sincere thanks to Prof. SHICHIROKU NOMURA for his kind guidance and valuable criticisms for this study. He is also indebted to Prof. ISAO MOTOMURA, Dr. GUNJI TOMITA, Dr. TAMETAKE NAGANO and Dr. KATSUHIRO OKADA for their kind suggestions.

MATERIAL AND METHOD

The daphnids were caught in the aquarium of the Institute. The eggs were removed from the brood chamber of the mother with two dissecting needles under the microscope. The transparency of the carapace enables us easily to observe the developmental process of embryos in the brood chamber. At room temperatures (20° C. ~25° C.), the embryo enters the nauplius stage, bursting the egg membrane, about eighteen hours after the deposition in the brood chamber. This membrane is tough. It bursts and breaks into elongate pieces, and are often found in the brood chamber together with the embryos which are to enter the nauplius stage (Fig. 1, b). Hatching takes place when the embryo moults the nauplius-membrane about thirty-eight hours after the deposition. The membrane is soft, thin and somewhat flexible (Fig. 1, d). In connection with this membrane,

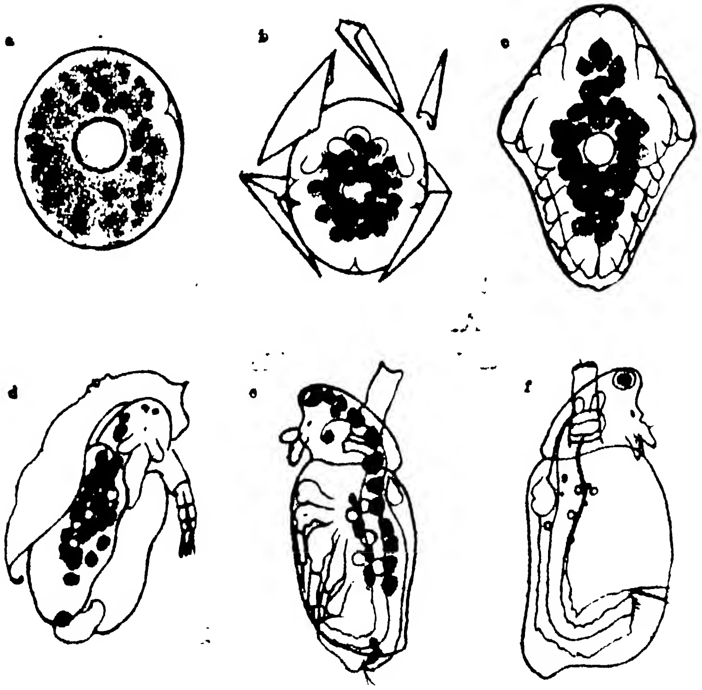

Fig. 1. Development of *Simocephalus vetulus*.

a. Gastrula (side view).
b. Bursting of the egg-membrane.
c. Nauplius (dorsal view).
d. Moulting of the nauplius membrane.
e. Hatched embryo (side view).
f. Released young (side view).

it may be mentioned here that the existence of a "pre-natal molt" was discussed by OBRESHKOVE and FRASER (1940) in some *Daphnia*. The embryo thus hatched is more or less motile, and yet stays in the brood chamber for about ten hours. The embryos are then released and become free-swimmers (1st instar young), fifty hours after the deposition.

The developmental process above mentioned could be traced more clearly in the eggs cultured in glass vessels with sterilized pond water than in the eggs developing in the brood chamber. The eggs thus cultured *in vitro* grow as fully, in almost the same period of time, as the ones developing in the brood chamber of the mother.

The measurement of respiratory quotient (R.Q.) was made on the four stages of development: gastrula, nauplius, hatched embryo and released young (Fig. 1; a, c, e and f). The embryological data are also given in Table 1.

For the measurement of the respiration, NOMURA's modification of THUNBERG's micro-respirometer was employed. The capacity of the respiratory chamber was 1.50 cubic centimeters, in which a small glass dish with capacity of about 0.1 cubic centimeters was supported on a tripod attached to the former. The volume of the capillary was 1.76 cubic millimeters per centimeter of the scale. The respirometer was placed, without shaking, in the thermostat regulated at 27°C. ± 0.005. Before measurement, usually ten minutes should have been allowed for the establishment of the temperature equilibrium of the respirometer. The shift of the index drop in the respirometer capillary was read every ten minutes during the experiment by means of a holizontal microscope with ocular micrometer. The movement of the index drop was observed to be uniform and regular for several hours. After each measurement, the eggs or embryos were examined microscopically and no appreciable changes during the experiment were found.

For the determination of oxygen consumption, 0.3 cc. of sodium hydroxide solution (2%) were put in the respiratory chamber to absorb carbon dioxide produced. The shift of the index drop in the capillary indicated the half volume of oxygen consumed actually by the animals, because the reduction in oxygen pressure in the chamber containing the animals was equally shared by the two chambers. In every measurement, ten eggs or embryos from the same brood were placed in the dish with 0.05 cc. of sterilized pond water. For the measurement of carbon dioxide production, ten different eggs or embryos of the same stage were used, and the respiration was allowed to proceed in the respirometer chamber without the sodium hydroxide solution. Then the shift of the index drop showed the difference of the volume of oxygen consumed and that of carbon dioxide produced. This value and the volume of oxygen consumed gave the volume of carbon dioxide produced. In measuring carbon dioxide production, 0.3 cc. of distilled water or the same volume of 5% sulphuric acid solution was placed in the respiratory chamber, instead of 0.3 cc. of sodium hydroxide solution. The movement of the index drop did not differ in both cases, during the preliminary experiment, but the water in the glass dish containing the eggs or embryos tended to evaporate when sulphuric acid solution was used. In the experiments, of which the results were reported here, 0.3 cc. of distilled water were used.

In calculating the volume of the gases, corrections were made for the water vapour tension and temperature. Respiratory data given in Table 1 were the average value of nine measurements. The present study was carried out from July to September 1948.

EXPERIMENT AND RESULT

The result of the experiment shows that the rates of oxygen consumption and carbon dioxide production per embryo per hour gradually increase. The rate of oxygen consumption of the released young (1st instar young) per individual per hour is 0.115 mm^3, and this value is in accord with the results obtained in experiments on the effect of temperature upon the oxygen consumption of the young of the same stage (paper in preparation).

a) Value of respiratory quotient. The respiratory quotient also increases gradually; 0.74 in gastrula, 0.80 in nauplius, 0.88 in hatched embryo and 0.99 in released young. This suggests that the energy source of development is mainly fat, in gastrula, with participation of protein or carbohydrate in some degree.

Table 1.

Changes in R.Q. in course of development of *Simocephalus*. For the details, see the text.

Period	1	2	3	4
Stage	Gastrula	Nauplius	Hatched embryo (in brood chamber)	Released young (free-swimmer)
Body length (mm)	0.240	0.352	0.458	0.624
Hours after egg deposition	10-12	25-28	42-45	55-60
O_2-consumption (mm^3) per embryo, per hr.	0.019	0.041	0.056	0.115
(O_2-CO_2) (mm^3) per embryo, per hr.	0.005	0.008	0.007	0.001
CO_2-production (mm^3) per embryo, per hr.	0.014	0.033	0.049	0.114
R.Q.	0.74	0.80	0.88	0.99

In the nauplius stage, the dominance of fat decreases and protein or carbohydrate increases the importance. In hatched embryo, the R.Q. is 0.88 and the rôle of carbohydrate as energy source is evident. In released young, it is obvious that carbohydrate plays the principal rôle of energy supply.

b) Relative respiratory rate. The relative respiratory rates were expressed as the volume of oxygen consumed and cabon dioxide produced per unit body volume, which was assumed as the cube of the body length. The rate in gastrula was taken as 100 per cent. Table 2 gives the results of the relative respiratory rates of the daphnid in course of the development.

Table 2.

Relative respiratory rates of *Simocephalus* in development.

Stage	Gastrula	Nauplius	Hatched embryo	Released young
Body length (mm)	0.240	0.352	0.458	0.624
Cube of body length	0.014	0.044	0.096	0.243
O_2-consumption (mm^3) per embryo, per hr.	0.019	0.041	0.056	0.115
O_2-consumption per unit body volume	1.375	0.932	0.583	0.432
Relative rate of O_2-consumption (%)	100.0	98.7	43.0	31.9
CO_2-production (mm^3) per embryo, per hr.	0.014	0.033	0.049	0.114
CO_2- production per unit body volume	1.000	0.750	0.519	0.469
Relative rate of CO_2-production (%)	100.0	75.0	51.0	46.9

The decrease in the relative respiratory rates continues with advance of the development. But the level of the rate of oxygen consumption falls more rapidly than that of carbon dioxide production. This is evidenced also in the increase of the respiratory quotient. In the previous report studied on the oxygen consumption of *Simocephalus* in the development until 10th instar, we pointed out that the egg must have showed a higher metabolic activity than the animals of the later stages (HOSHI, 1949). This supposition was supported evidently by the results obtained here.

DISCUSSION AND CONCLUSION

In embryos of various animals — *Fundulus* (AMBERSON and ARMSTRONG, 1933), *Melanoplus* (BOELL, 1935) and *Urechis* (HOROWITZ, 1940) — the changes of R.Q. indicated that carbohydrate was the principal energy source in the early development, in agreement with NEEDHAM's view. Attention is called, however, to the remarkable case where BRACHET (1934) found that the R.Q. at the segmentation stage in *Rana fusca* was about 0.7, whereas it approached unity during the gastrulation. He (1936) also observed further that the average value of R.Q. of the dorsal lip was 1.10 while that of the ventral part was 0.77. Employing a Cartesian diver BOELL *et al.* (1938) found also similar changes in amphibian embryos.

In unfertilized eggs of sea-urchin the value of R.Q. remained to be 1.00 or a little higher, but it fell greatly after fertilization (BOREI, 1934, '49). LINDAHL (1939) suggested that the respiration in early stage of *Paracentrotus* embryo must have been closely associated with the fat metabolism, followed by an increasing breakdown of carbohydrate. This view was supported by the results of ÖHMAN (1940), which showed that the R.Q. was 0.73 and 0.85 respectively at the two stages of 1 to 2 hours and 7 to 8 hours after fertilization. These findings were ascertained by his further studies which indicated that the total liqid contents by chemical analysis decreased gradually during 7 hours after fertilization (ÖHMAN, 1945).

The results of studies by BRACHET, LINDAHL and ÖHMAN, above mentioned, are opposed to NEEDHAM's view, and indicate the utilization of fat in the earlier stages and of carbohydrate in the later stages of development. The results of the present work also add a new case which is in disaccord with NEEDHAM's view.

WEISMANN (1877) and also BIRGE (1918) held that the embryos were nourished by mother in the brood chamber in which they were developing. The summer eggs of *Simocephalus*, in the brood chamber, absorb the yolk material gradually as the development proceeds (Fig. 1). The eggs cultured *in vitro* also develop and grow fully utilizing the yolk material, which suffices the development of the embryo to the free-swimming stage. RAMMNER (1933) and OBRESHKOVE and FRASER (1940) observed similar facts as the present results.

The clear cytoplasm of periphery of gastrula was found invaginated on one side of the embryo (Fig. 1. a). This process was clearly observed by the brief histological test. BOUIN's fluid or ten per cent formalin was employed in the fixation. Sections were cut ten μ in thickness. Most of these sections were stained with HEIDENHAIN's hematoxylin-eosin as usual. The results revealed that the invagination occurred at the prospective oral aperture on the ventral side. The cleavage of the egg was evidently found superficial. These findings are in agreement with the results reported by many workers in various kinds of the dephnids (DOHRN 1870; CLAUS 1876; GROBBEN 1879; WEISMANN and

ISHIKAWA 1887; LEBEDINSKY 1891; KÜHN 1908 and LUMER 1937).

In the center of the egg, a large oil drop is found (Fig. 1, a). The behavior of it will be studied in near future.

SUMMARY

(1) Respiratory quotients in the developing embryos of *Simocephalus vetulus* were measured at four stages, employing NOMURA's modification of THUNBERG's micro-respirometer.

(2) The value of R.Q. was 0.74 and 0.80 in the gastrula and in the nauplius stage respectively.

(3) The hatched embryo in the brood chamber showed the R.Q. of 0.88, which became 0.99 in released young (free-swimmer).

(4) The changes in R.Q. during development are closely associated with the absorption of the yolk material stored in the embryo, and suggest that fat plays the principal rôle as the energy source in the earlier stages of development, and carbohydrate in the later stages.

(5) The invagination of gastrula takes place at the prospective oral aperture on the ventral side. The cleavage of the egg is superficial.

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SCIENCE REPORTS OF THE TÔHOKU UNIVERSITY, 4TH SER.
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THE ECOLOGICAL INVESTIGATION CONCERNING THE RELATION BETWEEN THE CLIMATIC CONDITIONS AND THE POPULATION DENSITY OF THE RICE LEAF-MINOR, *AGROMYZA ORYZELLA*.[1]

BY

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(With 5 Text-figures)

(Received August 31, 1949)

INTRODUCTION

The rice leaf-minor, *Agromyza oryzella* MATSUMURA, is one of the most serious insect pests which injure the rice plant; it is distributed in the western half of Hokkaidô, the greater part of Northeast Honshû and the Hokuriku district. The said insect attacks the rice plants, mining in the mesophyll of leaves and appears two times during the growing period of the rice plant, *viz.*, the first generation in the nursery and the second in the paddy field.

In the present paper the relation between the population density of the said insect and its environmental meteorological conditions is dealt with.

The writer is much indebted to the late Prof. SANJI HÔZAWA for his many valuable advices and criticisms during the course of the present investigation. The writer is also grateful to Mr. HIROHARU YUASA, director of the Entomological Section of the Government Agricultural Experiment Station, for valuable suggestions.

LOCAL TYPES OF THE POPULATION DENSITY OF THE LEAF MINOR

The experiments were conducted at Akita in Akita Prefecture, Tateoka and

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[1] The results of the present study was reported in detail in Japanese in "Journal of the National Agricultural Experiment Station", Vol. 4, No. 1, 1948.

Fujishima in Yamagata Prefecture and Kurobe, Toyama and Tonami in Toyama Prefecture.

The larvae belonging to the first generation of this insect grow in the nursery attacking the leaves of the seedlings, and the second generation appears thereafter in the paddy field injuring the growing young plants. Thus the environmental conditions in the first and second generations of this fly are different. Therefore the population density of the 1st generation obtained in the nursery and that in the paddy field are not to be discussed biologically from the same point of view.

In the present experiments, therefore, the population density of the 1st and 2nd generation was measured in the especially prepared paddy fields of direct sown rice.

Two types of population density were recognized in this study (Fig. 1).

I. Population density of the 1st generation, *i.e.* the number of larvae, exceeds that of the 2nd generation. This is seen typically at Fujishima in Yamagata Prefecture.

II. The 2nd generation exceeds the 1st in population. This is observed at Akita Prefecture.

Thus the further investigations were conducted at the two representative localities, Fujishima and Akita above mentioned.

At Akita contrary to Fujishima, the larvae belonging to the 2nd generation are markedly more numerous than those belonging to the 1st. Concerning the 2nd generation, the environmntal resistance for the minor is not strict at Akita, but is severe at Fujishima. The number of larvae per one stump of the rice plant is 25–30 at Akita, but only 20 or so at Fujishima, and while the mortality is only 24 per cent at Akita, but it runs up to 52 per cent at Fujishima.

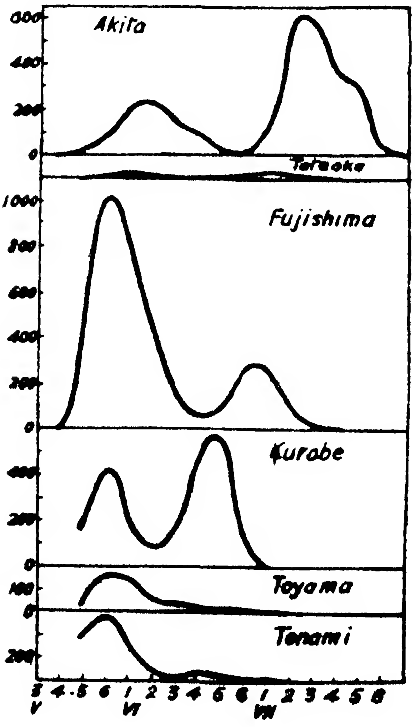

Fig. 1. Seasonal prevalence of the population density in the rice leaf-minor observed at several places in Honshû; represented by the number of larvae found in leaves of 20 stumps of rice plants.

THE ENVIRONMENTAL THERMO-HYGRO RELATION MEASURED AT TWO LOCALITIES

The air temperature and the relative humidity were measured by ASMAN's psychrometer during the period of the emergence of this leaf-minor.

It was concluded that the thermo-hygro relation of the environmental conditions is similar in the said two localities, and thus it seems that the thermo-hygro relation is not so important in the control of the population density of the leaf-minor.

It is recognized from this experiment that the environmental temperature is fairly low at Akita, and consequently, the environmental temperature must be investigated to clarify the limiting factor of the population density of the said insect.

THE TEMPERATURE LIMIT OF ACTIVITY OF THE LEAF-MINOR

The experiments were conducted at the temperature rising at the rate of 1°C in every 4 minutes using adult flies and mature larvae belonging to the 1st and 2nd generations (MOTOMURA, 1936; KATÔ, 1938).

Here, no significant difference was statisticaly recognized between the data obtained from the 1st and 2nd generations, or from the data obtained at Akita and at Fujishima; the results are tabulated below.

Table 1. Fiducial range of mean value of temperature, at which various conditions of activity are shown (Reliability 95%).

Condition of activity	Flies	Larvae
Slight movement	7.0-10.4°C	4.4-5.6°C
Standing on feet	9.1-12.8	—
Crawling	10.2-17.2	12.0-16.8
Normal activity	—	17.2-20.1
Flying	19.3-19.9	—
Nervous	29.0-30.2	26.4-31.4
Falling down (heat paralysis)	34.6-46.7	33.1-40.1
Death	40.9-46.7	42.4-46.5

The optimum zone of activity is in the range from 16.5 to 30.0°C. Therefore, normal activity is not observed below 15° nor above 30°C, and the flying begins at about 20°C.

In the case of the larval activity, the resistance of the temperature is observed below 14° and above 29°C. It seems therefore that the optimum temperature zone of activity of this insect is between 20° and 25°C, and consequently it is conceivable that the said insect is adaptable to a fairly low temperature environment.

MICRO CLIMATIC ENVIRONMENT OF THE LEAF-MINOR

The daily progress of the temperature in the mesophyll of the leaves in which the larvae of flies live, and of the leaf surface temperature and also of the

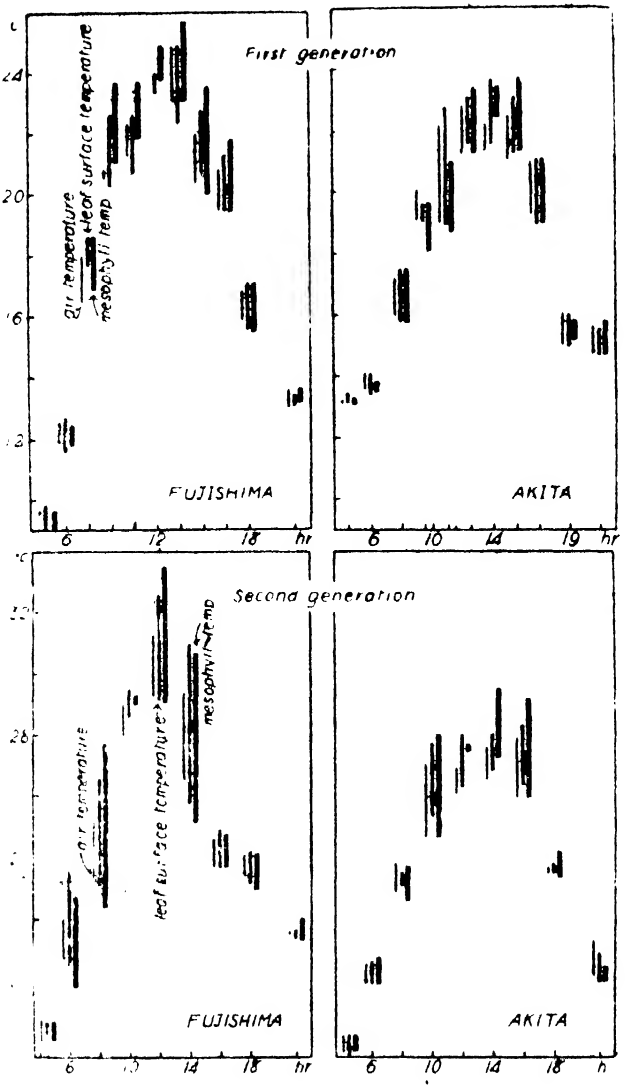

Fig. 2. The diurnal change of the temperature in the mesophyll of the leaves in which the larvae live, of the leaf surface temperature and of the air temperature adjacent to the leaf surface; length of bar shows the fluctuating range in temperature.

air temperature adjacent to the leaf surface were measured by thermocouples (Fig. 2).

At Akita, the temperature of the mesophyll is generally almost equal to the temperature of both leaf surface and air, but it is 1°C. or so higher than the other two only in the period from 10 a.m. to 4 p.m. At Fujishima, however, the leaf temperature is 1°C. or more higher than the other two already at 6 a.m. and 2°C. or more higher in the period from 10 a.m to 4 p.m. The phenomena above mentioned can be correlated to the diurnal rhythm of the macro-climatic conditions of the two localities (Fig. 3).

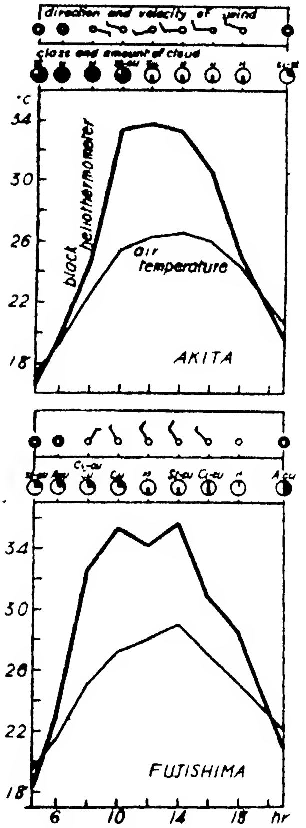

Fig. 3. A diurnal rhythm of the macro-climatic conditions measured in the period of the 2nd generation of flies.

At Akita the morning calm is observed and the haze in the morning weakens the solar radiant heat. On the other hand, this phenomenon is not seen at Fujishima and the absorption of the solar radiant heat is observed early in the morning.

THE VERTICAL DISTRIBUTION OF THE AIR TEMPERATURE

The vertical distributions of the air temperature in the nursery and also in the paddy field were measured in order to clarify the air temperature adjacent to the grass-crown of the rice plant where the flies live.

The lst generation (Fig. 4): Akita:- The fluctuation of the air temperature is from 13° to 24°C, having the modal range from 14° to 22°C. Fujishima:— The temperature fluctuates from 8° to 28°C and the mode of it covers the range from 14° to 24°C. Thus, it is clear that the temperature is fairly high at Fujishima.

When the micro-environmental temperature of the leaf minor and the temperature reaction of the said insect are taken into consideration, it is recognized that at Fujishima the temperature range is optimum for the activity of this insect, while at Akita the environmental temperature is too low.

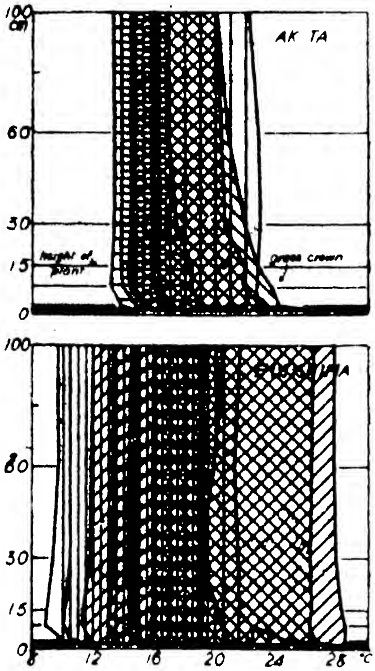

Fig. 4. The vertical distribution of the air temperature in the nursery measured during the period of the 1st generation of flies. The vertical distributions of the minimum and maximum temperature obtained every day during the examination were drawn upon each other. Thus the temperature fluctuation during the examination and the modal range of the same are clearly shown.

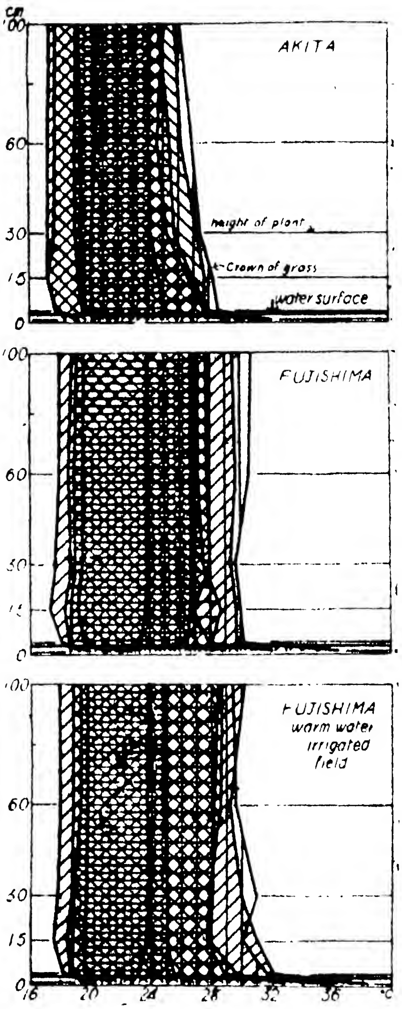

Fig. 5 The vertical distribution of the air temperature in the paddy field during the period of the 2nd generation of flies.

The 2nd generation (Fig.5): Akita — The environmental temperature changes from 18° to 27°C and the modal range is from 19° to 26°C. Fujishima — The fluctuation of the environmental temperature is from 19° to 32°C and the mode of it is from 20° to 30°C.

Therefore, at Fujishima the activity of the said insect seems to be inhibited by the high temperature, and acceleration of activity by optimum temperature environment is seen at Akita.

CONCLUSION

It was recognized that the environmental temperature is most effective upon the seasonal prevalence of the population density of the rice leaf-minor, *Agromyza oryzella* MATS., and that the local type of emergence of this insect seems to be related with the local type of temperature environment.

From the investigation concerning the temperature reacion of the activity of the said insect, it was concluded that the optimum temperature zone of activity of this insect is between 20° and 25°C.

The diurnal change of temperture in the mesophyll of the leaves in which the larvae of the flies live and the air temperature adjacent to the grass-crown of the rice plant where the flies are living, were measured at Akita in Akita Prefecture and Fujishima in Yamagata Prefecture. From these expeiments it was clarified that there is a distinct local type in the so-called "micro-environment", corresponding to the local type of the emergence of flies.

Consequently it was concluded that the local type of emergence of the rice leaf-minor depends mainly upon that of the temperature environment.

POST SCRIPTUM

The abstract of this investigation was read at 18th International Congress of Entomology in Stockholm (1948).

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COMPOUND EYES OF *CULEX PIPIENS* VAR. *PALLENS* COQUILLETT. (MORPHOLOGICAL STUDIES ON THE COMPOUND EYE IN THE MOSQUITO NO. I)

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(With 3 Figures and 1 Plate)

(Received August 31, 1949)

INTRODUCTION

Although there are many reports on the compound eye of insects, only BUDDENBROCK (1937) has investigated that of the mosquito. Therefore, the writer has attempted to clear up the structure of the eye of the mosquito with special reference to photic reaction

The writer herein describes the results of investigation on a Japanese common mosquito, *Culex pipiens* var. *pallens* COQUILLETT.

This work was supervised by Prof. Dr. MUTSUO KATÔ, to whom the writer is indebted. The writer also acknowledges his thanks to Dr. TAMETAKE NAGANO, and Assist. Prof. Dr. GUNJI TOMITA for their help.

MATERIAL AND METHOD

The specimens used for this investigation were obtained in the neighbourhood of the Biological Institute.

For histological observations, specimens between twelve and twenty four hours after emergence were used. In order to obtain the light-adapted eyes, the insects were placed near the window of the laboratory under diffused day-light for four or five hours. Then they were killed and fixed. For the killing and fixing, some fixative mixtures (CARNOY's fliud, alcoholic BOUIN's fluid, and

KAHLE's fluid) and hot water were employed. The best result was obtaind with KAHLE's fixative. Hot water was very convenient, especially for fixing in the dark room, but was disadvantageous because the histological structure seemed to be considerably injured thereby.

After embedding with the paraffin method, their heads were cut in the thickness from 5μ to 10μ. Depigmentation, where desired, was accomplished in GRENACHER's mixture. For the staining, DELAFIELD's haematoxylin with eosin was especially useful, HEIDENHAIN's iron haematoxylin alone or with light green, and MALLORY's triple connective tissue staining were also employed.

For the dark-adapted eye, the insects were placed in a dark room for from three to twelve hours, and then killed mostly with hot water and sometimes with the fixative. Then the specimens were treated in the same manner as described above.

EXTERNAL STRUCTURE

I. Shape of the Compound Eye

In the mosquito the outer surface of the compound eye is apparently distorted egg-apple-shaped (Fig. 1), and is considerably different in the male and female; namely, the second antennal segment, so-called "*torus*", is larger in the male than in the female; consequently, the concave line of the eye surface which contacts with *torus* is deeper in the former than in the latter (Fig. 2).

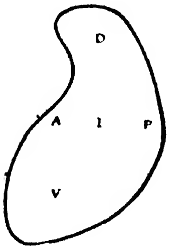

Fig. 1. Diagram of external surface of a compound eye (left eye of female). D, dorsal; V, ventral; A, antero-lateral; L, lateral; P, postero-lateral.

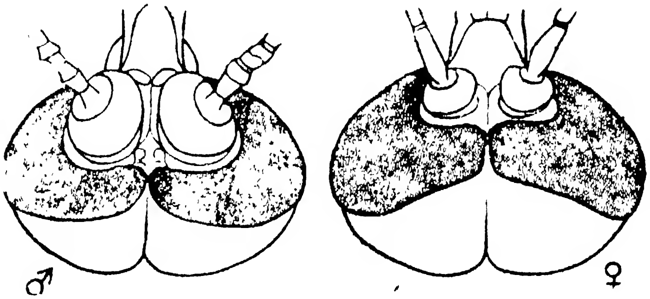

Fig. 2. Male and female heads under the same magnification (dorsal view), showing differences in size of "*torus*", and in shape of compound eye. × ca. 50

The head breadth and the maximum distance between right and left eyes in the frontal portion of the head were measured using 5 male and female specimens, as shown in Table 1.

Table 1. Confidence limits of means of head breadth and maximum distance between right and left eyes in frontal portion of the head (in 95% reliability).

	Male	Female
Head Breadth (mm.)	0.74~0.81	0.81~0.86
Maximum Distance between Both Eyes (mm.)	0.44~0.50	0.37~0.40

The significance of the difference between male and female was tested by the method of ***Analysis of Variance*** and was found to be negative for the head breadth, but on the contrary, highly positive for the maximum distance between both eyes.

II. Surface Area and Facet-Number of the Compound Eye

For the measuring of the surface area of the compound eye and the number of the corneal facets composing one compound eye, the entire cornea, which was stripped off from the head with sharpend needles and mounted in glycerin-alcohol, was drawn on section paper with aid of an ABBE's camera lucida under a constant magnification, and then, the number of the corneal facets were counted one by one, and also the approximative area of the surface was estimated. These treatments were applied to the 20 eyes obtained from the above-mentioned 10 specimens. The results are shown in Table 2.

Table 2. Confidence limits of surface area of compound eye and number of the corneal facets composing one compound eye (in 95% reliability).

	Male	Female
Surface Area (μ^2)	210570~236030	283185~325065
Number of Facets	440~462	503~560

From the above-mentioned results, very highly significant difference between the male and female was clarified statistically; namely, both the surface area of the compound eye and the number of the corneal facets are larger in the female than in the male.

III. Size of the Facet

For the study of the size of the facet the whole surface of the eye was divided into the following five portions, *viz.*, 1) dorsal, 2) ventral, 3) lateral 4) antero-lateral, 5) postero-lateral (Fig. 1), thus, the total surface area of 25 facets belonging to each of these portions was measured three times by the above mentioned manner, then, the average area of one facet in each portion and the diameters of an inscribed circle of the hexagonal facet were estimated. These measurements were applied to two of the compound eyes obtained from the male and female.

The results are shown in Table 3. The dimensional characters of the facet become small in the following order; namely, ventral, dorsal, antero-lateral, lateral, and postero-latersl portion.

Table 3. Confidence limits of means of dimensional characters of facets (in 95% reliability).

Sex	Portion	Surface Area (μ^2)	Diameter (μ)
Male	Postero-Lateral	275~405	19.2~20.2
	Lateral	374~466	20.7~23.3
	Antero-Lateral	431~549	23.2~24.2
	Dorsal	476~550	23.4~25.2
	Ventral	6.5~675	27.1~27.8
Female	Postero-Lateral	264~462	17.7~23.1
	Lateral	463~575	23.1~25.7
	Antero-Lateral	484~602	23.6~26.4
	Dorsal	576~636	26.1~26.5
	Ventral	656~754	28.3~28.5

INTERNAL STRUCTURE

The ommatidia vary in size not only in diameter but also in length, showing that the confidence limit of the mean of length is 54.1~57.3μ in 95-per cent reliability. The cellular composition of each ommatidium is well understood from comparison of the longitudinal and transverse sections (Fig. 3).

I. Ommatidium in the Light-Adapted Eye

(a) *The corneal part.* This part is composed of two layers, the cornea and the lens. The cornea is the thin layer forming the facet of the ommatidium and is externally hemispherical. The thickness of the cornea is about 1μ. The lens, the shape of which is biconvex, shows interesting differences of structure between light-adapted and dark-adapted eyes as described later, therefore the writer intends to divide the corneal part into the cornea and the lens, though it has been usually considered that "cornea" and "lens" are synonymous.

(b) *The iris part.* Beneath the lens, there is a group of four crystal cells

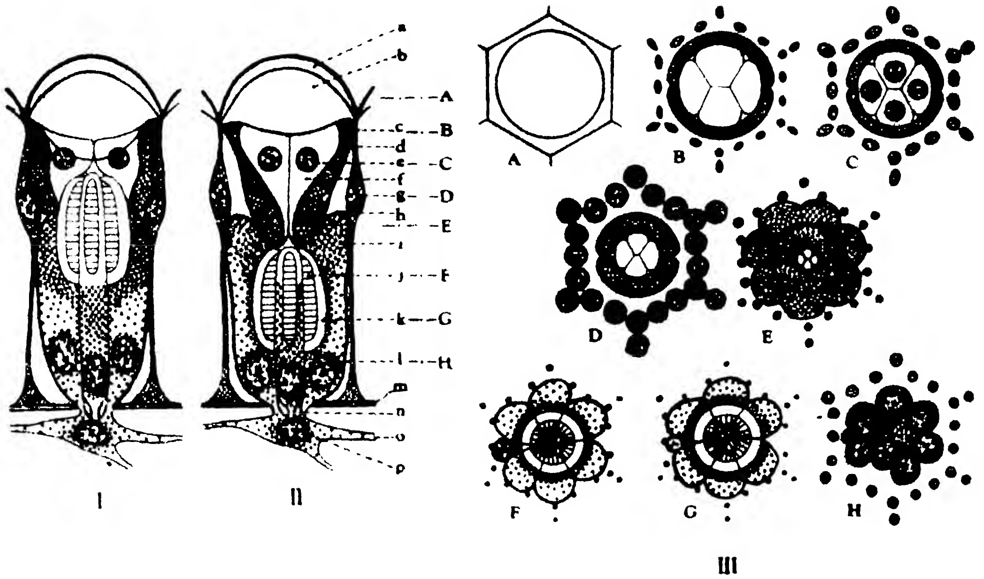

Fig. 3. Diagram of light- and dark-adapted ommatidia.
(I). Longitudinal section of an ommatidium from dark-adapted eye.
(II). Longitudinal section of an ommatidium from light-adapted eye. The eight capital letters at the right of the figure indicate the levels at which the sections for figure (III) were taken. (III) Transverse sections of light-adapted ommatidia.
a, cornea; b, lens; c, nucleus of iris pigment cell; d, iris pigment cell; e, nucleus of crystal cell; f, crystal cell; g, nucleus of accessory pigment cell; h, accessory pigment cell; i, retinular cell; j, rhabdom; k, Schaltzone (intermediate zone); l, nucleus of retinular cell; m, basement membrane; n, nerve fibre; o, hypobasal pigment cell; p, nucleus of hypobasal pigment cell.

forming a cone-like body. The proximal end or apex of the cone-like body does not quite diminish to a point, but after becoming very slender it is slightly enlarged again.

The cone-like body is seen corresponding with crystalline cone of "eucone-type eye" (GRENACHER, 1879; EXNER, 1891; PHILLIPS, 1905; HESSE, 1908; ZIMMERMAN, 1914; OGUMA, 1917; JOHNAS, 1924; NOWIKOFF, 1934; AINO, 1933, '34, '35, '36; SUGIYAMA, 1933; UCHIDA, 1934; UMBACH 1934; COLLINS, 1934; YAGI, 1938; DAY, 1941), or with pseudocone of "pseudocone-type eye" (GRENACHER, 1879; DIETRICH, 1909; BEDAU, 1911), but the cone-like body apparently consists of the four cells themselves, since there is not found any secreted crystalline substance in them, and their nuclei are generally observed in the central part of each cell. Judging from the above, it may be said that the compound eye of this insect belongs to "acone-type eye", which was first recognized by GRENACHER (1879).

Surrounding the cone-like body, there are two densely pigmented cells, the iris pigment cells. The distal parts of the iris pigment cells are rather thin, but gradually thicken proximally having the proximal end which become suddenly thin again. The nuclei of the iris pigment cells are always situated at their distal end, either in light- or dark-adapted conditions.

(c) *The retinular part.* This part is composed of a group of usually eight pigmented retinular cells, which are highly differentiated on inside portion of each of them. The arrangement of the retinular cells of this insect is more similar to that of the water-boatman, *Notonecta*, (GRENACHER, 1879; BEDAU 1911) than many other insects which have eucone eyes; namely, at the distal portion of the retinula seven retinular cells envelop another retinular cell, and then one of these seven surrounding cells becomes slender proximally, and thus the central cell, inversely, becomes more thick. The nuclei of these cells are situated at the level of the proximal end of the cell except the central cell in which the nucleus is commonly found at the middle or rather distal level of the retinula. The diameter of these nuclei is about 7μ.

Details of this part will be given in the description of the following three areas, *viz*, rhabdom, cytoplasmic portion, Schaltzone (intermediate zone).

The rhabdom seems to be a transparent solid or of crystalline structure, at least, in the living state, but in the stained preparation it is deeply stained with some dyes (eosin, safranin, HEIDENHAIN's iron haematoxylin, etc.), and observed to consist of eight rhabdomeres.

The cytoplasmic portion contains black pigment granules and the nuclei of the retinular cells. The pigment granules, in this portion, are sparsely distributed throughout the cytoplasm, but at the parts surrounding the rhabdom or the nuclei they are far more abundant than the others. The pigment granules contained in the distal portion of the central retinular cell are densely distributed in the axial cytoplasmic space of the rhabdom. Although this pigment is apparently a part of the retinular pigment, the writer especially intends to call it "rhabdom-pigment" because of its characteristic movement caused by change of the photic environment as described below. The part containing the rhabdom-pigment shows a crescent shape in transverse section (Plate V, 4), and a rod-like shape in longitudinal section (Plate V, 3). Its distal end reaches the level of approximately two-thirds of the entire length of the rhabdom.

The intermediate zone between the rhabdom and the densely pigmented layer of the cytoplasmic portion is called Schaltzone (HESSE, 1901). In transverse section treated with suitable fixing and staining method, the Schaltzone shows a fine fibrillar structure. These fibrils seems to be the neuro-fibrils which are the terminations of the optic nerve-fibre coming through the basement membrane from the optic ganglion, as suggested by HESSE (1901) and AINO (1933).

(d) *The hypobasal part.* In the depth of the eye there is a transverse septum, known as the basement membrane. Beneath the basement membrane, there is a large heavily nucleated cell which contains pigment granules. It is here called "hypobasal pigment cell". The branches of the hypobasal pigment cells form a thin pigment-layer between the eye and the brain.

(e) *The interommatidial part.* Surrounding each of ommatidia, there are extremely elongated pigment cells, known as the accessory pigment cells. The distal end of each cell attaches to the periphery of a cornea, and the proximal end reaches the basement membrane. The nuclei are always found in the distal portion of the cells; to express more precisely, at the almost middle level of the cone-like body in the iris part. The number of the accessory pigment cells, surrounding each ommatidium, is more or less varied but usually eighteen.

No evidences of tracheal tapetum as found in the moth eye (JOHNAS, 1924; SUGIYAMA, 1933; COLLINS, 1934; UMBACH, 1934; YAGI, 1938; DAY, 1941), of tracheal sac as in dragon-flies (GRENACHER, 1879; EXNER, 1891; ZIMMERMAN, 1914; OGUMA, 1917; AINO, 1933, '34 '36), and of reflecting pigment as in mantis (HESSE, 1908; ZIMMERMAN, 1914; AINO, 1935) or grass-hopper (UCHIDA, 1934), were found.

II. Ommatidium in the Dark-Adapted Eye

The feature of the ommatidium in the dark-adapted condition is distinctly different from that in the light-adapted condition.

The rhabdom, pushing and opening the crystal cells laterally, moves into the iris part, and the lens thickens proximally, so that the distal end of the rhabdom and the proximal edge of the lens draw near each other until they almost near touch. Owing to the entering of the rhabdom into the iris part, the shape of the cone-like body which was shown in the light-adapted eye, disappears. In addition, the iris pigment cells contract distally and open aside to some extent; consequently, the pupil of the ommatidium becomes markedly enlarged in comparison with that of the light-adapted eye.

In the condition of the said ommatidium, the rhabdom-pigment is not seen, but the proximal portion of the central retinular cell; namely, the portion between the basement membrane and the proximal end of the rhabdom, becomes to contain densely the pigment granules. Thus the crescent-shaped pigment mass which was seen in the light-adapted eye completely disappears (Plate V, 5, 6).

Other changes recognized by the writer in the feature of the ommatidium are that the proximal portion of the retinula becomes a little slender, and that the nuclei in the retinular cells distally moves very slightly except that in the central retinular cell.

In order to compare the distance of migration of the rhabdom and the rate in change of the thickness of the lens in the light-adapted eye with those in the dark-adapted eye, five well-sectioned individuals were selected from light- and dark-adapted insects, and next, five ommatidia well sectioned longitudinally were selected from each of the said individuals. From each of the selected 50 ommatidia, the thickness of the lens was compared with the diametre of the same, and also the distance from the basement membrane to the proximal end of the rhabdom was compared with the entire length of the ommatidium. The results are shown in Table 4.

Table 4. Confidence limits of means of b/a and d/c in light- and dark-adapted eyes (in 95% reliability).

	Light-Adapted Eye		Dark-Adapted Eye	
b/a	.39	.43	.57	·63
d/c	.17	.20	.35	.37

a, diametre of lens; b, thickness of lens; c, entire length of ommatidium (from basement membrane to distal end of cornea); d, distance from basement membrane to proximal end of rhabdom.

From these measurements the highly significant difference between light- and dark-adapted eyes was recognized statistically.

In both processes of the light- and dark-adaptation, although the migration of the rhabdom is almost completed during the time from ten to twenty minutes the time required for the completion of the change of the features in the lens in the iris part, and in the rhabdom-pigment, is so long as to count about from two to three hours.

DISCUSSION

BUDDENBROCK (1937) described that the total diameter of five facets of the European mosquito, *Culex pipiens*, is 80μ. Threfore it is easily considered that the diameter of one facet is approximately 16μ. But the writer's measuring of the facet of var. *pallens* shows that it ranges generally between 18μ and 28μ.

According to EXNER (1889, 1891), two types are recognized in the compound eyes of the insect; most of the insects active in daytime have the apposition-eye and most of the insects active at night or in dim light have the superposition-eye. In the latter, the pigment granules contained in the ommatidium migrate according to the change of the light intensity, consequently the apposition-image is formed in the light, and the superposition-image is formed in the darkness or dim light.

But in the compound eye of the mosquito, from the structual point of view, the superposition-image is never formed either in light or in dark environment. but the apposition-image is formed. Nevertheless by changing the structure of the ommatidium, which depend mainly upon the migration of the rhabdom, this insect is probably able to adapt to any photic environment correspondingly.

It would be very interesting to know the mechanism of the photic adaptability in the case of this insect which is conspicuously different from that in many other insects as have been known hitherto (Parker, 1932).

SUMMARY

1 The Japanese common mosquito *Culex pipiens* var. *pallens* COQUILLETT, was investigated.

2. The shape of the surface of the compound eye seems almost like a distorted egg-apple. The concave line of the shape which contacts with *torus* is deeper in the male than in the female.

3. The surface area of the compound eye and the number of facets composing one compound eye, both are larger in the female than in the male.

4. Size of the facets are different according to their position on the surface of the compound eye; their order from the large to the small is on ventral, dorsal, antero-lateral, lateral, and postero-lateral.

5 The characterestics of the ommatidium are as follows. 1). It belongs to "acone-type eye". 2) Its length is considerably short in comparison to the diameter of the corneal facet. 3). The rhabdomeres are of short rod-like shape. 4). No evidences of tracheal tapetum, of tracheal sac, and of reflecting pigment, were found.

6. In the dark-adapted eye, 1) the rhabdom moves distally and enters the iris part, 2) the lens thickens, 3) the pupil in the iris part expands according to the contraction and transformation of the iris pigment cell, 4) the rhabdom-pigment disappears from the rhabdom, 5) the proximal portion of the retinula becomes slightly slender.

7. By reason of the fact that the lateral face of the rhabdom is surrounded by a densely pigmented layer both in, the light- and dark-adapted eyes, the writer considers that the apposition-image is always formed in the eye of this insect.

8. The migration of the rhabdom caused by change of the photic environment becomes almost complete in a considerably short time (10~20 minutes). However

it requires a comparatively long time (120~180 minutes) until each of the complete light- and dark-adapted eye forms its own characteristic cellular structure.

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EXPLANATION OF PLATE V

Photographs from the cmpound eyes of *Culex pipiens* var. *pallens* COQUILLETT. 1 and 2 were taken from a preparation which had been killed in light-adapted state, depigmented in

Grenacher's mixture, and stained with *Delafield's* haematoxylin and eosin. 3 to 6 were taken from the preparations which had been slightly stained with same dyes without depigmentation.

1. Frontal section of head (female). × 70
2. Longitudinal section of compound eye. × 600
3. Longitudinal section of light-adapted eye. × 600
4. Transverse section of light-adapted eye through the retinular part, mostly. × 500 Note the presence of the crescent-shaped mass of the rhabdom-pigment.
5. Longitudinal section of dark-adapted eye. × 600
6. Transverse section of dark-adapted eye through the retinular part, mostly. × 500 Note the absence of the rhabdom-pigment in the rhabdom.

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STUDIES ON THE VEGETATION OF MT. ZAÔ*

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(With one Figure)

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Mt. Zaô stands between latitude 38° 0'–38° 10' N on the borderland between Miyagi and Yamagata Prefectures in Northeastern Japan. The mountain consists of several peaks, namely Myôgamine (1490m), Kumanodake (1840m), Kattadake (1759m), Sugigamine (1745m), Byôbudake (1817m) and Fubôsan (1705m). The former three peaks are in the northern part and have an extensive bare area of recent volcanic ejecta, while the latter three which are in the southern part are old extinct volcanoes now clad with dense vegetation. The general aspects of the vegetation and the succession of plant communities were studied from the standpoint of plant sociolgy.

The writer wishes to thank Prof. Y. YOSHII for his supervision during this study.

I. GENERAL ASPECTS OF THE VEGETATION

The characteristic features of the plant communities developing on the volcanic ejecta of Mt. Zaô was studied by Prof. Y. YOSHII (1940) who pointed out that the complex structure of the vegetation was due to volcanic activity. Three vertical regions in vegetation, as is usual on high mountains throughout the northern half of Japan, can also be seen on Mt. Zaô. These comprise the deciduous forest region, the subalpine coniferous forest region, and the alpine scrub and dwarf scrub region.

A. The deciduous forest region.

The mountain-side from 500 to 1100m above sea level is largely occupied by

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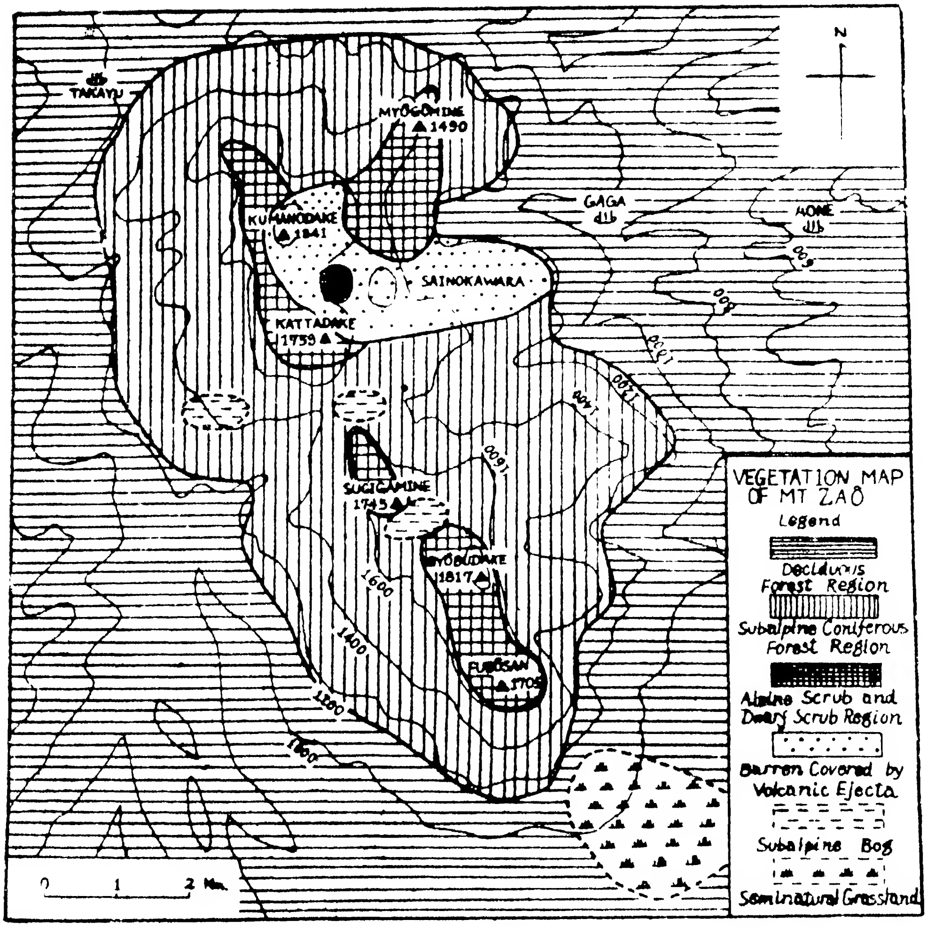

Fig. 1.

deciduous forests, the lower limit is uncertain because of human interference. The climatic climax community in this region is a forest dominated by the Japanese beech, *Fagus crenata* and other communities are largely subclimax ones due to human agency or edaphic condition.

The following communities are distinguished in the region:

1. *Fagus crenata* community. This community once occupied an extensive area in the region, but was diminished gradually by fire and lumbering. This beech forest is a typical climatic climax community of the montane deciduous forest region throughout Northeastern Japan. The structure of the community is as follows:

Tree layer: *Fagus crenata* d, *Quercus crispula* o, *Acer mono* var. *eupictum* o, *A. japonicum* o; Shrub layer: *Sasa kurilensis* la, *Lindera umbellata* o; Field layer:

Maianthemum dilatatum var. *nipponicum* f, *Disporum smilacinum* o, *Trientalis europea* var. *eurasiatica* o, *Mitchella umbellata* o. *)

2. *Fagus crenata-Quercus crispula* community. This is regarded as a developing community on volcanic ejecta or as a secondary community regenerating after lumbering. Thus, it is perceived that the community will migrate into the climatic climax, a pure forest of *Fagus crenata*, in the future. The floral composition of this community is more complex than the climax beech forest; it shows the following structure.

Tree layer: *Fagus crenata* d, *Quercus crispula* d, *Kalopanax pictum* o, *Magnolia ovbovata* o, *Acer mono* var. *eupictum* o, *A. japonicum* o, *Carpinus Chonoskii* o, *C. laxifolia* o, *Betula Schmidtii* o, *Alnus tinctoria* var. *glabra* o, *Fraxinus Sieboldiana* var. *serrata* o; Shrub layer: *Tritomodon campanulatum* f, *Hydrangea paniculata* var. *floribunda* o, *Clethra barbinervis* o, *Lindera umbellata* a, *Sasa kurilensis* a; Field layer: *Desmodium racemosum* o, *Aster ageratoides* subsp. *japonica* o, *Solidago japonica* o, *Pertya Koribanum* o, *Disporum similacinum* o, *Maianthemum dilatatum* var. *nipponicum* f, *Astilbe Thunbergii* o, *Thalictrum tuberiferum* o.

3. *Quercus crispula* community. This community is a pure forest of young oak which developed on a repeatedly lumbered area, that is, the community is a typical secondary forest in the deciduous forest region. The community shows the following structure:

Tree layer: *Quercus crispula* d, *Acer rufinervi* o, *Fraxinus Sieboldiana* var. *serrata* f, *Meisteria campanulata* f, *Sorbus commixta* o; Shrub layer: *Clethra barvinervis* o, *Ilex Sugeroki* o, *Rhododendron Albrechtii* o, *Rh. quinquefolium* o, *Menziesia ciliicalyx* var. *multiflora* o, *Sasa kurilensis* la; Field layer: *Tripterigium Regelii* f, *Disporum smilacinum* f, *Maianthemum dilatatum* var. *nipponicum* f, *Pirola incarnata* o, *Shizocodon soldanelloides* o, *Hugeria japonica* o.

4. *Miscanthus sinensis-Zoysia japonica* community (Seminatural grassland). This grassland is a result of lumbering and burning of the natural forest by man and it is now in an arrested stage of development due to repeated burning and mowing. Although the community is dominated with perennial grasses and dwarf bamboos, young trees are also found in abundance. An example of the floral composition is as follows:

Upper field layer: *Miscanthus sinensis* d, *Arundinaria ramosa* a, *Pleioblastus Chino* a, *Lespedeza Buergeri* f, *Spodiopogon silvericus* o, *Serratula deltoides* o, *Hydrangea paniculata* var. *floribunda* a, *Weigela hortensis* o; Lower field layer: *Zoysia japonica* ld, *Scabiosa Fischeri* var. *japonica* a, *Astilbe Thunbergii* f, *Pteridium*

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*) r=rare, o=occasional, f=frequent, a=abundant, d=dominant, la=locally abundant.

aquilinum var. *japonicum* a, *Ixeris dentata* o, *Aster ageratoides* subsp. *ovatus* o, *Lysimachia japonica* o.

B. The subalpine coniferous forest region.

This region occupies the area from 1200 to 1600 m and is dominated by a subalpine fir, *Abies Mariesii*, accompanied with abundant birch, *Betula Ermanii* var. *communis*. Bogs are also found in this region especially on the flat undrained sites where trees can not establish themselves.

The distinct communities found in this region are the following:

1. *Abies Mariesii* community. This forest community occurs extensively over the region maintaining themselves for a long time by natural reproduction. Since *Abies Mariesii* is the most shade-enduring tree in the region, it can establish itself even in the deep shade of the forest floor. The birch on the contrary is a typical light demanding tree and can establish itself only in an open place. The structure of the community is as follows:

Tree layer: *Abies Mariesii* d, *Betula Ermanii* var. *communis* f, *Sorbus commixta* o; Shrub layer: *Sasa kurilensis* ld, *Viburnum furcatum* f, *Rhododendron Albrechtii* f, *Acer Tschonoskii* o, *Sorbus commixta* o, *Ilex Sugeroki* o; Field layer: *Abies Mariesii* o, *Plagiogyra Matsumureana* o, *Maianthemum dilatatum* var. *nipponicum* o, *Stretopus japonicum* o.

2. *Betula Ermanii* var. *communis* community. This is a pure stand of birch which developed as a secondary forest or as a pioneer one on open area. The community occurs throughout the subalpine region and stretches therefrom to the deciduous forest region downwards and to the alpine scrub and dwarf scrub region upwards. An example of this community shows the following structures:

Tree layer: *Betula Ermanii* var. *communis* d, *Quercus crispula* o, *Abies Mariesii* r, *Fagus crenata* r; Shrub layer: *Sasa kurilensis* d, *Rhododendron Fauriae* var. *roseum* form. *rufescens* o; Field layer: *Solidago japonica* o, *Gentiana Makinoi* o, *Pedicularis yezoensis* o, *Galium japonicum* o, *Tripterigium Regelii* o, *Viola Selkirki* o, *Maianthemum dilatatum* var. *nipponicum* o.

3. *Fauria Crista-galli-Sieversia pentapetala* community (Subalpine bog). Bogs are found on undrained, flat places where snow is heavy, and primary bogs which developed on volcanic ash as already verified by Prof. Y. YOSHII (1931) on Mt. Hakkôda are recognized. The structure of the bog community is remarkably different from place to place owing to the sudden change of conditions, but the following structure is seen frequently:

Field layer: *Fauria Crista-galli* d, *Sieversia pentapetala* a, *Rhyncospora Yasudana* a, *Carex omiana* var. *monticola* f, *Narthecium asiaticum* f, *Eriophorum gracile* o, *Coptis trifolia* o, *Drosera rotundifolia* o, *Loiseleuria procumbens* o, *Aletris* *foliata* o; Moss layer: *Sphagnum* sp. a.

C. The alpine scrub and dwarf scrub region.

Above the subalpine coniferous forest region scrub and dwarf shrub communities occupy an area from 1600 m to the summits of the peaks (1700–1840 m). These communities are also seen occasionally on ridges and recently laid volcanic ejecta in the upper part of the subalpine coniferous forest region where trees can not establish themselves owing to unfavourable habitat conditions. The following communities are distinguishable in this region:

1. Alpine scrub community. Alpine scrubs occupy the greater part of the alpine region except for where they cannot establish themselves. Two distinct types are recognized in the scrub communities, viz. a dwarf pine, *Pinus pumila*, community and a dwarf alder, *Alnus Maximowiczii*, community.

a. *Pinus pumila* community. This community is dominated with *Pinus pumila* accompanied by several shrubs; it occurs usually on drier habitats. An usual structure of the community is as follows:

Shrub layer: *Pinus pumila* d, *Acer Tschonoskii* a, *Sorbus commixta* f, *Viburnum furcatum* f, *Taxus cuspidata* o, *Ilex Sugerokii* o, *Betula Ermanii* var. *communis* o, *Salix Reinii* o, *Rhododendron Fauriae* var. *roseum* form. *rufescens* o; Field layer: *Hydrangea paniculata* var. *floribunda* o, *Solidago japonica* o, *Viola Selkirkii* o, *Maianthemum dilatatum* var. *nipponicum* o.

b. *Alnus maximowiczii* community. This is a community of deciduous shrubs of which *Alnus Maximowiczii* is dominant. Coniferous shrubs on the contrary are scarcely found. The community occurs on a snowy scree and alongside a brook where the soil is moist and drainage and aeration are good. It usually has the following floral composition:

Shrub layer: *Alnus Maximowiczii* d, *Sorbus commixta* o, *Acer Tschonoskii* o, Field layer: *Sasa kurilensis* a, *Veratrum stamineum* var. *glabrum* o, *Viola Selkirkii* o, *V. acuminatum* o, *Percarpa circaeoides* o, *Plagiogyria Matsumureana* o.

2. *Empetrum nigrum* var. *japonicum*-*Loiseleuria procumbens* community. This dwarf shrub community occurs around the summits of peaks in the alpine region where prevailing winds are strong. It is also found on newly laid lava flows in the subalpine coniferous forest region as well as in the alpine region. The community is formed of the following components:

Field layer: *Empetrum nigrum* var. *japonicum* d, *Loiseleuria procumbens* d, *Vaccinium Vitis-Idiaea* f, *Arcteria nana* f, *Maianthemum dilatatum* var. *nipponicum* f, *Tilingia ajanensis* o, *Shizocodon soldanelloides* o, *Lycopodium obscrum* o, *Alnus Maximowiczii* o, *Spiraea betulifolia* o, *Menziesia ciliicalyx* var. *multiflora* o, *Juniperus communis* var. *nipponica* o.

II. PLANT SUCCESSION ON VOLCANIC EJECTA.

An extensive area on the eastern sides of Kumanodake and Kattadake is covered by a lava flow and the summits of the northern peaks, viz. Myôgômine, Kumanodake and Kattadake, with volcanic ejecta. This volcanism is said to have occured about seven hundred years ago. The lava flow which is called "Sainokawara", occupies an area between 1600 m to 1100 m and its greater part still remains barren, although a few plants are scattered on its surface. In this region are found communities in various seral stages of development. Although the floral composition of an early seral stage is similar everywhere on the lava flow, continued development gradually tends to make different communities according to both altitude and habitat. The following types were found in the course of succession owing to altitude.

A. Lower end of lava flow.

The lower end of the Sainokawara lava flow, 1000-1200 m above sea level, is clad by a relatively dense vegetation in comparison with the upper parts, and the floral components of the plant communities differ considerably from those of the upper parts. The following seral stages are seen in the communities developing in this place:

1. *Maackia amurensis-Miscanthus sinensis* stage. The community of this stage occurs at the inner portion of the area where the lava is thick and previous vegetation was completely annihilated. It is a scrubby community composed of the following plants:

Shrub layer: ***Maackia amurensis*** a, ***Weigela hortensis*** f, ***Salix vulpina*** f, ***Quercus crispula*** o, ***Menziesia ciliicalyx*** var. ***multiflora*** o. ***Palura pilosa*** o, Field layer: ***Miscanthus sinensis*** a, ***Carex Wrightii*** a, ***Maianthemum dilatatum*** var. ***nipponicum*** f, ***Sanguisorba albiflora*** f, ***Aletris foliata*** o, ***Solidago japonica*** o, ***Trientalis europea*** o, ***Ixeris dentata*** o, etc.

2. *Quercus crispula-Weigela hortensis* stage. The community of this stage is composed of stunted *Quercus crispula* and a number of shrubs, and shows a scrubby appearance. The following is an example of the structure of this stage:

Subtree layer: ***Quercus crispula*** a, ***Maackia amurensis*** o, ***Clethra barvinervis*** o, Shrub layer: ***Weigela hortensis*** f, ***Menziesia ciliicalyx*** var. ***multiflora*** f, ***M. pentandra*** o, ***Hydrangea paniculata*** var.. ***floribunda*** o; Field layer: ***Maianthemum dilatatum*** var. ***nipponicum*** a, etc.

3. ***Quercus crispula*** stage. At the very end of the lava flow, where the lava is thin and where some plants survived disaster, there is an oak forest

which may belong to the *Quercus crispula* stage. There the oak trees attain a hight of about ten meters and form a forest with a dense canopy. The following structure is seen:

Tree layer: *Quercus crispula* d, *Fraxinus Sieboldiana* var. *serrata* a, *Acer rufinervi* o, *A. japonicum* o, *Clethra barvinervis* f, *Tritomodon campanulatum* o; Shrub layer: *Menziesia ciliicalyx* var. *multiflora* f, *Palura pilosa* f, *Viburnum furcatum* f, *Rhodbdendron Albrechtii* o; Field layer: *Maianthemum dilatatum* var. *nipponicum* f, *Ilex Sugerokii* o, *I. crenata* o, *Rhododendron Kaempferi* o.

From the foregoing results it may be said that the oak forest is brought out as a primary forest passing through the scrub stages. The oak forest merges into a beech forest, the climatic climax community of the deciduous forest region, as the soil becomes mature.

B. Main part of lava flow.

The main part of the Sainokawara lava flow ranges from 1200 m to 1600 m in altitudes, and a community of *Abies Mariesii* and *Betula Ermanii* var. *communis* occupies the mature soil of the surrounding area. The lava where thick remains almost undecomposed, therefore its vegetation is so pauce that scarcely ten per cent of the area is occupied by plants as yet. We can find the following stages in the communities developing there:

1. Bare stage. The greater part of the lava flow is still bare and only a few non-exacting plants are found, *viz.*, *Deschampsia flexuosa*, *Reynoutria japonica*, *Solidago japonica* and *Salix Reinii*.

2. *Empetrum nigrum* var. *nipponicum-Loiseleuria procumbens* stage. On the slopes and marginal portions of the lava flow, where the lava is thin as compared with flat places, dense carpets of dwarf shrub plants with invading tree seedlings are found. As an example the following floral composition is shown:

Field layer: *Empetrum nigrum* var. *nipponicum* d, *Loiseleuria procumbens* d, *Pinus pentaphylla* o, *Juniperus communis* var. *japonica* o, *Vaccinium hirtum* r, *Spiraea betulifolia* r, *Solidago japonica* r, *Shizocodon soldanelloides* r, *Calamagrostis Langsdorfii* r.

3. *Pinus pentaphylla* stage. Near the margin of the lava flow, where the lava is thin and the climax community was left close by, there occurs a scrubby community mainly of *Pinus pentaphylla*. This pine is a tree which originally inhabits ridges in the transition zone between the deciduous forest and the subalpine coniferous forest regions, and its abundant occurrence is due to its non-exacting and easily dispersible characters. This scrub community is just like that of *P. pumila*, an alpine dwarf pine, in appearance. The structure of the community is as follows:

Subtree layer: *Pinus pentaphylla* a; Shrub layer: *P. pentaphylla* t; Field layer: *Empetrum nigrum* var. *nipponicum* d, *Loiseleuria procumbens* a, *Ledum palustre* var. *nipponicum* o, *Lycopodium obscrum* o, *Gaultheria Miqueliana* o, *Salix Reinii* o, *Rhododendron Tschonoskii* var. *pentaphyllum* o, *Spiraea betulifolia* r, *Sanguisorba albiflora* r.

The development of the plant community begins on a bare area later invaded by *Deschampsia flexuosa*, *Reynoutria japonica*, etc. (the bare stage), and through the next stage or the alpine dwarf scrub dominated by *Empetrum nigrum* var. *nipponicum* and *Loiseleuria procumbens* (the *Empetrum nigrum* var. *nipponicum* and *Loiseleuria procumbens* stage) the community transforms into the scrubby community of *Pinus pentaphylla* (the *Pinus pentaphylla* stage). Athough the climatic climax community of the summits is the scrub of *P. pumila*, the scrub of *P. pentaphylla* prevails for a long time as a proclimax community.

III DIFFERENCE IN FLORAL COMPOSITION BETWEEN THE NORTHERN AND THE SOUTHERN PEAK GROUPS

Wide bare areas extend over the northern peaks which erupted repeatedly until recently, while a dense vegetation covers the southern peaks where volcanic activity ceased long ago. For such reasons a significant difference is recognized in the floral composition between both peak groups, though a considerable number of plants are in common.

1. The following plants occur only on the northern peaks:

Hieracium japonicum, *Tripterigium Regelii*, *Dicentra peregrina* var. *pusilla*, *Reynoutria japonica* var. *compacta* form. *colorans*, *Chondradenia Fauriae*, *Pinguicula vulgaris*, *Ledum palustre* var. *nipponicum*, *Phyllodoce aleutica*, *Maackia amurensis* var. *Buergeri*, etc.

2. The following plants are found exclusively on the southern peaks:

Potentilla Matsumurae, *Halenia corniculata*, *Ligusticum acutifolium*, *Helacleum nipponicum*, *Anemone narcissiflora*, *Chrysanthemum rupestre*, *Saussurea brachycephala*, *Patrinia palmata* var. *gibbosa*, *Geranium yezoense* var. *japonicum* subvar. *glabrum*, *Thymus quinquecostatus* var. *laxus*, *Primula modesta* var. *Fauriae*, *Bupleurum nipponicum*, *Bistorta nitens*, *Miscanthus Matsumurae*, *Juniperus chinensis* var. *Sargentii*, *Rosa acicularis* var. *nipponensis*, etc.

3. The following plants are found on both peak groups:

Empetrum nigrum var. *nipponicum*, *Vaccinium Vitis-Idaea*, *Diplycosia adenothrix*, *Loiseleuria procumbens*, *Arcterica nana*, *Spiraea betulifolia*, *Menziesia ciliicalyx* var. *multiflora*, *Juniperus communis* var. *nipponicum*, *Salix Reinii*, *Gentiana Makinoi*, *Shizocodon soldanelloides*, *Anaphalis margaritacea*, *Solidago japonica*, *Aletris foliata*, *Maianthemum dilatatum* var. *nipponicum*, *Veratrum Maximowiczii*, *Lycopodium sabinaefolium* var. *sitchense*, etc.

Among the plants which occur exclusively on the northern peaks *Hieracium*, *Reynoutria*, *Tripterigium* and *Dicentra* are most characteristic. They are pioneer plants which invade bare volcanic ejecta and can endure poverty and dryness of soil. They are, however, crowded out by dwarf shrubs or herbaceous plants as the soil become mature.

The majority of the plants which are restricted to the southern peaks are perennial herbs of mesophytic character composing an alpine meadow. Those plants which grow on both peak groups are widespread species with non-exacting and easily dispersible characters, that is they are dwarf shrubs and xerophytic herbs.

SUMMARY

1. The vegetation of Mt. Zaô in Northeastern Japan was studied from a synecological standpoint, and plant succession on volcanic ejecta was also investigated.

2. The vegetation is divided into three regions according to the physiognomies of the climax communities, *viz.* the deciduous forest region (500–1200 m), the subalpine coniferous forest region (1200–1600 m) and the alpine scrub and dwarf scrub region (above 1600 m). The structure of each community in each region was analysed in detail.

3. Plant succession on the Sainokawara lava flow, which was ejected more than seven hundred years ago, is so slow that the greater part of the lava field is still bare. Closed communities are found on the end or the margin of the lava flow.

4. As to the seral communities developing on the lava flow four distinct stages are distinguished, *viz.* bare, dwarf scrub, scrubby and forest stages.

5. Some xerophytic herbs occur restricted to the recently erupted northern peaks, while mesophytic herbs are exclusive to the southern peaks. Dwarf shrubs are found on both peak groups.

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BOTANICAL STUDIES OF BOG LAKES IN A VOLCANIC REGION WITH SPECIAL REFERENCE TO LACUSTRINE BACTERIA.[1]

PART III. MICROBIAL POPULATION.

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In the preceding papers (JIMBÔ, 1949) of this series I have outlined the characteristic features of the pool water and the bottom mud, which are the very habitats of lacustrine bacteria. This one deals with the bacterial population of this bog lake. An account of its fungus flora of special interest is also given. All the experiments regarding the micro-organisms were confined to materials from the deepest centre of Naga-Numa bare of vegetation (2.5 m. in depth).

OCCURRENCE OF COMMON BACTERIA

First, I will mention the findings in regard to the occurrence of common bacteria, that is to say, those capable of thriving on ordinary plates.

The Number of Common Bacteria in Water. One dull afternoon in the middle of August of 1941 the bacterial numbers in the surface and bottom waters were counted by the use of the plate method with sodium-caseinate agar medium (*vide* WAKSMAN and FRED, 1922). The bottom water was taken by means of the EKMAN sampler thoroughly washed with the bottom water itself. After 16 days' incubation at room temperature (15 to 20°), the following numbers were obtained: Bacteria —including actinomycetes— per c.c. of the pool water reach

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1) Contributions from the Mt. Hakkôda Botanical Laboratory. No. 34. The cost of this investigation was in part defrayed by a grant from the Scientific Research Expenditure of the Department of Education. The writer expresses his gratitude to the Department of Education.

1,370 in the surface layer and 960 near the bottom. The latter number corresponds to 70 per cent. of the former. It is of interest to note that 44 per cent. of the bacteria in the surface water and 34 per cent. of those in the bottom water give rise to pigmented colonies, of which over 70 per cent. are yellow, no less than 10 per cent. orange, another 10 and odd per cent. pink and the rest are violet or light green. In contrast to the great abundance of chromogenic bacteria in the lake water, they are absent in the bottom mud as will be seen later. Some workers have already pointed out that pigmented bacteria are plentiful in waters whilst they are scarcely found in soil. This fact was regarded as related to the light penetration. The first men to notice the predominance of pigmented bacteria in the lake water were SNOW and FRED (1926). According to them, the bacterial population of the water of Lake Mendota (Wisconsin) was made up of 52 per cent. white and cream-coloured bacteria, 35 per cent. yellow and orange, and 11 per cent. pink and red, only a small percentage of it being brown, fluorescent green, violet and black. GRAHAM and YOUNG (1934) found in the water of Flathead Lake in the Rocky Mountains (Montana) that coloured bacteria represented 39 per cent. of the total bacteria and were dominated by yellow and orange forms, pink and red being less common and violet, blue and black rare. Moreover, according to HENRICI (1939), chromogenic bacteria are few in number in the brown water of dystrophic lakes, such as Mary Lake and Helmet Lake[1] in Wisconsin. On the other hand, HENRICI and McCOY (1938), who worked with the bottom mud of Lake Mendota, an eutrophic lake, reported that the mud also contained pigmented bacteria in somewhat smaller numbers than the water, and that, thus, the proportion of pigmented bacteria varied from 10 to 17 per cent. in depths down to 18 cm., below which there was a marked reduction. They were for the most part yellow and orange, red being fewer. The nearer the surface the more were the coloured bacteria. Furthermore, the muds of the dystrophic lakes mentioned above were found to be strikingly poor in chromogenic bacteria.

Again in 1947, six years after the above counts were made, and for the third time in 1949, the bacterial number was counted about noon on a clear day at the end of August of 1947 and at the beginning of July of 1949 in exactly the same manner as in 1941. 550 and 1,220 bacteria per c.c. of the surface water were counted in the respective years. As many as 55 and 17 per cent. of them were pigmented, of which 88 and 46 per cent. were yellow, 8 and 49 per cent. pink and

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[1] Their colours are said to be 123 and 233 respectively on the platinum-cobalt standard.

4 and 5 per cent. orange. Shake cultures in test-tubes just as made of the mud to be mentioned in the following paragraph gave roughly 200 of anaerobes per c.c. of the surface water. A point of importance to be noted is that none of the anaerobes in the water, too, was coloured.

The Number of Common Bacteria in Mud. In the same afternoon as the cultivation was made from the water, benthic bacteria in the bottom mud taken by means of the EKMAN-BIRGE dredge were counted. The plate method with the sodium-caseinate agar medium was used for the aerobes. Anaerobes were cultivated in a deep layer of the agar medium mentioned in a test-tube overlain by a thick layer of plain agar. The temperature and the period of incubation were the same as above. One gram of the dried mud taken from the surface of the bottom harbours 1,011,500 bacteria capable of growing on plates—inclusive of actinomycetes. It is to be noted that pigmented bacteria present in the water in so great a proportion are absent in the mud (see above). Anaerobes did not admit of an accurate estimation by this method, a rough estimate of their number being 100,000. They were all devoid of pigments. The anaerobic test-tube cultures showed a marked gas evolution and gave out a putrid smell.

Six and eight years afterwards, at the same time when counts were made of bacteria in the water again, I found, by the use of the plate method, 91,670 and 824,742 bacteria per gram of the dried superficial layer of the bottom mud and 83,250 and 636,364 bacteria in the deeper part of it. At this time, too, pigmented bacteria could not be found in the mud. The numbers of anaerobic bacteria developed in the shake cultures were several ten thousands both in the upper and lower layers of the mud, all of them being colourless.

In conclusion, the points to be noted can be summarized in the following:

1. Especially interesting is the fact emerging from the foregoing data that pigmented bacteria are confined to the water altogether lacking in the mud, nearly half of the bacterial flora of the water being made up of chromogenic species.

2. On the other hand, anaerobic bacteria in the water are also devoid of pigments.

3. As has been seen already, the bottom mud has a capacity of intensely absorbing oxygen, so that the water permeating the mud[1] is undoubtedly free from oxygen. In the light of these facts bacteria living therein are thought to be either obligatory or facultative anaerobes, which latter will be represented by the plate counts. Above figures obtained by the shake cultures, however, are far lower than those to be expected, due probably to the unsatisfactory method of

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1) The water content of the deeper part of the mud was found to be 86.7 per cent.

counting.

4. It appears, at all events, that the lacustrine pigmented bacteria are all aerobic.

5. Consideration of the above points leads us to suppose that the pigmentation is linked in some way or other with the bacterial respiration, though the previous workers have laid stress on the light factor.

6. There is no doubt whatever that the bacterial flora in the water is, for the most part, distinct from that in the bottom mud.

The results obtained in 1947 are noticed to be excessively low in numerical respects when compared with those recorded six years ago and two years later. And the same can be said of the fungi (see below). At present I cannot account for such divergences, within the limited scope of this investigation, without requisite observations taken over a number of years to trace the variation of the microbial population in the course of time.

PERIPHYTIC BACTERIA IN WATER

For direct observation of the bacteria in waters, HENRICI (1933) and KARSINKIN (1934) put forward the submerged slide method. Briefly the method consists in keeping slides submerged in lake water for an appropriate period and then staining and examining the bacterial cells coating their surfaces—periphytic bacteria or bacterial periphyton1). The former worker regarded the number of bacterial cells per day per square millimetre of the surface area of the slide as some measure for the bacterial number in water. This conception was based on the view stressed notably by ZOBELL and ANDERSON (1936) that aquatic bacteria tend for the most part to grow attached to suspended matters due to an accumulation of dissolved nutrients on their surfaces by adsorption2). However, HENRICI's claim is considered to be open to criticism, as will be seen later.

An attempt was made to count the periphytic bacteria in Naga-Numa and to compare the result with the plate numbers, which is described above. Two batches of slides were lowered in the centre of Naga-Numa, one just below the surface—at a depth of 15 cm.—and the other deeper down at a depth of 2 m., that is to say, $\frac{1}{2}$ m. distant from the bottom. After a period of 9 days, towards

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1) For a detailed treatment of this method, the reader is referred to HENRICI (1936).

2) *See also* ZOBELL and ALLEN, 1935; HOTCHKISS and WAKSMAN, 1936; KUSNETZOWA, 1937; STARK, STADLER and MCCOY, 1938; HEUKELEKIAN and HELLER, 1940. It is worth noting also that HENRICI and MCCOY (1938) found much less bacteria on slides buried in the bottom mud than those submerged in the overlying water, presumably due to the fact that the mud particles had already provided them with an immense surface.

the end of August of 1941, the slides were taken out, quickly dried and stained with rose bengale (1 in 100 of a 5 per cent. solution of phenol). Bacterial cells fixed on the slides were rods of diverse sizes and shapes, frequently associated with each other in chains or in clumps, evidence that a multiplication had taken place on the glass surface. Among them were cells only faintly tinted with stain. They seemed to be no longer viable and sooner or later broken down. The numbers of the periphytic bacteria were 2,550 at a depth of 15 cm. and 92 at 2 m. per day per sq. mm. In the bottom water there were periphytic bacteria very poor in comparison with the surface water, the ratio being 1 : 27. There is a startling contrast between this and that obtained by the plating method. This latter showed an admittedly different ratio of 7 10 as mentioned above.

Reverting to HENRICI's experimental data (HENRICI, 1936; 1938; 1939), it is to be noted that actually did he, too, observe very frequently a falling off in the number of periphytic bacteria as the depth increased. Thus he recorded a striking fall in their number beneath the thermocline in stratified lakes of varied types in Wisconsin and Minnesota. To give notable examples: In Mary Lake and Helmet Lake (Wisconsin), which are dystrophic waters lying in *Sphagnum*-bogs (19 and 10 m. deep), the periphytic bacteria were plentiful near the surface but almost absent from the hypolimnion at the end of June, when the water temperature was 25° at the surface and sank even to 4° and 6° respectively in the hypolimnion. Nevertheless, this was not reflected in plate counts. He did advance the conclusion that the water temperature exerts a very marked effect on the vertical distribution of periphytic bacteria. Similar results were obtained in Lake Glubokoje by KARSINKIN. The plate counts, on the other hand, are known to show, as a rule, no definite tendency in the vertical distribution of bacteria in the lake water. HENRICI stated as to it that in plate counts inactive bacteria are also taken into account to give higher counts for the hypolimnion. But this statement does not give a lucid explanation. The situation is far more complicated by the intervention of bacterial multiplication on the glass surface.

It is more likely that the submerged slide method does give not the actual number of bacterial cells present in the water but the bacterial activity. Submerged slides offer anew a surface, as it were, with an infinite area, compared with minute suspended particles, on which bacteria settle and multiply freely thriving upon nutritive substances concentrated on the glass surface in the course of the submersion. Here the rate of multiplication is undoubtedly related to the water temperature to a great extent, the bacterial growth being promoted by a higher temperature in the warm epilimnion to give a much greater bacterial number per day per sq. mm. than the cool hypolimnion where a lower temperature slows down the growth rate. During the above period of my experimentation the temperature of the surface water might reach 25° or so at midday, while the bottom water was, in all probability, to remain as cool as about 15°, so that in the latter the bacterial multiplication, that is, the bacterial activity was greatly reduced by the lower temperature there. At all events, a source of fallacy might arise when depending very much upon the submerged slide method so helpful in certain other aspects. In reality HENRICI did rightly mention that the slide count is related to the bacterial activity, but ventured to rely upon it in assessing the bacterial number.

OCCURRENCE OF PHYSIOLOGICAL GROUPS OF BACTERIA

Making use of the same samples of the surface water and the bottom mud—both from the upper and lower layers of it—as taken for the bacterial counts in 1947, attempts were made to obtain selective cultures of a variety of physiological groups of bacteria, viz., (1) aerobic non-symbiotic nitrogen-fixing bacteria, (2) anaerobic non-symbiotic nitrogen-fixing bacteria, (3) nitrite-forming bacteria, (4) nitrate-forming bacteria, (5) denitrifying bacteria, (6) thiosulphate bacteria, (7) sulphate-reducing bacteria, (8) aerobic cellulose-decomposing bacteria, (9) anaerobic cellulose-decomposing bacteria.

Methods. To determine whether *Azotobacter* is present, ASHBY's solution (*see* FRED and WAKSMAN, 1928, p. 20) placed in a shallow layer in Erlenmeyer flasks was inoculated with the water or mud sample. As a test for *Clostridium*, a deep layer of WINOGRADSKY's solution (WINOGRADSKY, 1902) in large test-tubes inoculated with the sample was incubated after pasteurized previously at 80° for 10 minutes. For nitrite- and nitrate-forming bacteria were used WINOGRADSKY's solution (*see* FRED and WAKSMAN, 1928, p. 22) and FRED-DAVENPORT's solution (FRED and DAVENPORT, 1921) respectively, run as a shallow layer into Erlenmeyer flasks. The cultures were tested for ammonia and nitrites for the former bacteria and for nitrites and nitrates for the latter from time to time over a period of incubation. The presence of denitrifying bacteria was confirmed by inoculating the samples into test-tubes filled with GILTAY's solution (*see* FRED and WAKSMAN, 1928, p. 25) and testing at proper intervals for ammonia, nitrites and nitrates, gas evolution being also taken into account. In the test for thiosulphate bacteria, BEIJERINCK's solution (*see* FRED and WAKSMAN, 1928, p. 28) was used for *Thiobacillus thioparus* and WAKSMAN's solution (WAKSMAN, 1922*a*) for *Th. thiooxidans*, Erlenmeyer flasks being here employed. The test for sulphate-reducing bacteria was carried out by the use of VAN DELDEN's solution (VAN DELDEN 1904) with which stoppered bottles were entirely filled. For aerobic cellulose-decomposing bacteria the samples were inoculated into DUBOS' solution (DUBOS, 1928) with which test-tubes were partly filled and into which a strip of filter-paper was in part immersed. For anaerobic ones test-tubes were filled with OMELIANSKI's solution (OMELIANSKI, 1902), the filter-paper being wholly dipped therein.

The tests for ammonia, nitrites and nitrates consisted in colour reactions with NESSLER's reagent, TROMMSDORF's reagent and diphenylamine reagent, respectively (*see* FRED and WAKSMAN, 1928, p. 56 *et seq.*).

As a rule, the cultures were incubated at about 20° for three weeks. It has been mentioned already that the water temperature at the deepest bottom may lie at about 15° even in midsummer. Hence this incubation temperature is obviously above the temperature at the bottom of the pool.

Results Obtained. Out of a number of bacterial groups tested for, only the denitrifying bacteria was found to be present not in the mud alone but also in the water, there being no evidence that any of the rest inhabited the water or mud. This indicates that the biochemical changes due to bacteria are on the whole inactive in this pool

FUNGUS FLORA

While lake bacteriology has gone to some extent, little attention has hitherto been paid to the fungus population of lakes. The mycological aspect of a lake necessarily involves two questions: What becomes of fungus spores brought on the lake surface by wind or incorporated into the lake water with the drainage water? Is there any active fungal growth capable of effecting changes to speak of in a lake? Quantitative and qualitative studies of the fungus flora in lakes are the necessary first steps to an understanding of the fate of the fungal contaminations and constitute an approach to the solution for the problem of whether fungi lead an active existence in the lake. My work in this line presented here throws some light on the presence of some fungi active in the mud.

Filamentous fungi were counted in the same mud sample as used for the bacterial count. Plate counts made of fungi after 4 days' incubation at room temperature (18–24°) using WAKSMAN's acid medium (WAKSMAN, 1922*b*) gave 36,550 fungi per gram of the dried surface layer of mud in 1941.

In the years 1947 and 1949, the fungus flora, too, was reinvestigated together with the bacterial flora mentioned above. The water gave 7 and 8 of fungi per c.c. 1,157 and 2,268 fungi were counted per gram of the dried surface layer of mud, whilst in the deeper layer there was found no fungus whatsoever. This latter fact would naturally be expected in view of the oxygen deficiency in the mud, where fungi which are, as a rule, aerobic are unable to exist.

On identifying the fungus species developed on the plates, I noticed an interesting fact. In the bottom mud the fungus flora is restricted a great deal and dominated by a few forms, whereas a great variety of species are found in the water, no particular fungi being numerous. The dominant organisms in the mud are a species of *Mortierella* belonging to the Mucorales but characterized by the lack of columellae, and a blue-green species of *Penicillium*. As many as 28 per cent. of fungus colonies developed on the plates were found to be those of *Mortierella* and 25 per cent. the penicillium, both together being roughly half that of the total colonies. The remainder of the fungus colonies were represented by a limited number of fungus species, that were present only in small numbers without exceeding 4 per cent. Such, for example, is the case of *Trichoderma*. It would seem likely that the colonies of fungus species other than the two predominating ones, say *Trichoderma*, were derived merely from quiescent spores which had fallen on the pool surface and deposited on the bottom at a relatively recent time since which no much time had ever elapsed to destroy their germinating capacity. These fungi are unlikely to be distributed in the mud as active mycelia: Direct microscopic examination of the mud yielded no information in mycological respects.

In contrast with the preponderance of certain forms in the mud, the water contains a fungus flora rich in species without one or more particular fungi being dominant. This is the case with the air over the pool, too, much the same colonies as from the water being seen on the plates exposed to the air. Such a close resemblance between the pool water and the atmosphere regarding the fungus flora would seem to admit of no doubt that the fungi in the water of this pool are, for the most part, to be regarded as mere spore contaminations from the air.

Reverting to the distinctive fungus population of the surface layer of the mud, consideration of the predominance of the two species of fungi mentioned and the consequent paucity of other forms, unlike the wealth of fungi in the water, leads me to suppose that the mortierella and the penicillium are tolerant of the oxygen deficiency to some extent, whereby they may attain a growth in the mud fitting into a more or less anaerobic existence. In default of experiments on their behaviour to varying oxygen concentration, it, of course, is impossible to make further statements. But in the light of these facts it would seem clear that certain fungi may maintain themselves in the bottom mud of a lake.

Yeasts, including those giving rise to red colonies prominently displayed among others, were met with on the acid plates for the common fungi inoculated with the water or the mud. That they are air-borne was ascertained by exposure of sterile plates to the air on the pool.

Search was made for aquatic fungi of Saprolegniaceae and myxobacteria in the surface layer of the bottom mud. In every case, however, negative results were given. To test whether the aquatic fungi are present, a small quantity of tap-water with the body of an insect was run into each test-tube and inoculated with the mud after sterilization. In the course of a week no development of saprolegniaceous fungi occurred. For the test for myxobacteria, hare droppings were placed in Petri dishes lined with wet cotton-wool for the sake of secured humidity, sterilized and inoculated with a suspension of the mud. In spite of a prolonged incubation, no fruiting bodies of myxobacteria appeared upon the droppings. In view of these facts it seems likely that none of the organisms mentioned is present in these pools.

Finally, I would like to mention something of the results emerging from an inquiry conducted for fungus strains possessing antibiotic properties among those isolated from the water and the mud of this pool. Out of no less than 30 fungus cultures of a variety of species, two strains of *Trichoderma* were found to exert a marked bacteriostatic action against both *Staphylococcus aureus* and *Escherichia coli*. Besides them, a number of both gram-positive and gram-negative bacteria as well as acid-fast ones tested are also inhibited their growth. It is now under investigation whether it is due to gliotoxin known to be characteristic of *Trichoderma* and some others (*vide* WAKSMAN, 1947), the result of which will be detailed in the future.

In concluding this third part I desire to offer my thanks primarily to Prof. Dr Y. YOSHII for his kindly help throughout the investigation. I gratefully acknowledge Prof. Dr M. KUROYA and Dr N. ISHIDA for their unstinted help as regards the inquiry for antibiotics. I also wish to record my indebtedness to Prof. Dr T. KAWAMURA and to Prof. Dr M. UENO of the Ōtsu Hydrobiological Station for permission to work in the library there. Opportunity may here be taken to express thanks to the late Dr S. YOSHIMURA, whose loss we mourned recently, for a great deal of help since there was commenced my study of lake bacteria.

SUMMARY

1. Plate counts gave 1,370, 550 and 1,220 bacteria, including actinomycetes, per c.c. of the surface water in summer-time of three different years, and, on the other hand, 1,011,500, 91,670 and 824,742 per gram of dried superficial mud on the deepest bottom, somewhat less bacteria beneath.

2. While a moiety of bacteria in the pool water are pigmented, mostly yellow, chromogenic bacteria are altogether absent from the mud deprived of

oxygen, whose presence is, in all probability, indissolubly connected with the pigmentation.

3. The number of periphytic bacteria declines markedly with the depth down to 1/27 of that at the surface, whereas plate counts of the surface and bottom waters show a ratio of 10:7. It follows from this, that the submerged slide method refers largely to the bacterial activity.

4. Apart from denitrifying bacteria, various groups of bacteria tested for are found to be lacking. At all events, therefore, biochemical changes brought about by bacteria are on the whole inactive in this pool.

5. The fungus population of the mud confined to the surface layer is dominated by a species of *Mortierella* and a penicillium, presumably tolerant to some extent of the oxygen deficiency, unlike the rich fungus flora in the water which reflects that in the overlying air. It would seem likely that the two forms of fungi mentioned are more or less active in the mud, while the others as well as those in the water and the air are considered to be merely spore contaminations.

6. Out of a variety of fungus strains isolated, two of *Trichoderma* were found to produce a bacteriostatic substance.

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ACTION POTENTIAL AND CONDUCTION OF EXCITATION IN THE LEAF OF *MIMOSA PUDICA*.

BY

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(With 7 Text-figures)

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On the action current or the action potential in cells and tissues of plant, there are many interesting studies such as on *Nitella* and on sensitive plants as *Mimosa*, and the applicability of the law of excitation as in the nerve or the muscle has been proved. Among others *Mimosa pudica* has been studied by many investigators in connection with the interesting phenomena that the leaves react rapidly to stimuli and its reaction is not only confined to the stimulated part but also can be conducted through the whole plant. Various theories have been advanced on the mechanism of the conduction of stimulus or excitation. Recently the action potential (negative change) which accompanies the conduction of excitation in *Mimosa* was proved by BOSE and UMRATH, and it is suggested that the conduction occurs by the propagation of protoplasmic excitation, just as in the case of nervous impulse in animals.

The writer has investigated the excitation and its conduction in the leaf of *Mimosa pudica*. The results herein described concern the pattern and the nature of action potential led off at various parts of leaf and the general manner of excitatory conduction.

The apparatus used in recording of the potential change consists of the string galvanometer (EDELMANN's small type) or the electromagnetic oscillograph (YOKOGAWA's N-3 type with H-type vibrator) (Fig. 1) and the direct current amplifier. The oscillograph is used to record simultaneously the potential changes of two points of the plant. The electrodes used to lead off the action potentials

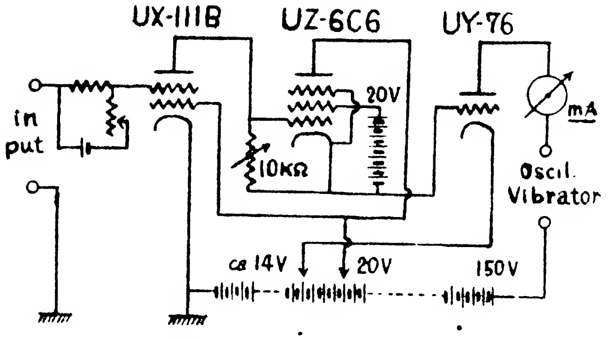

Fig. 1. Arrangement of the amplifier for electromagnetic oscillograph.

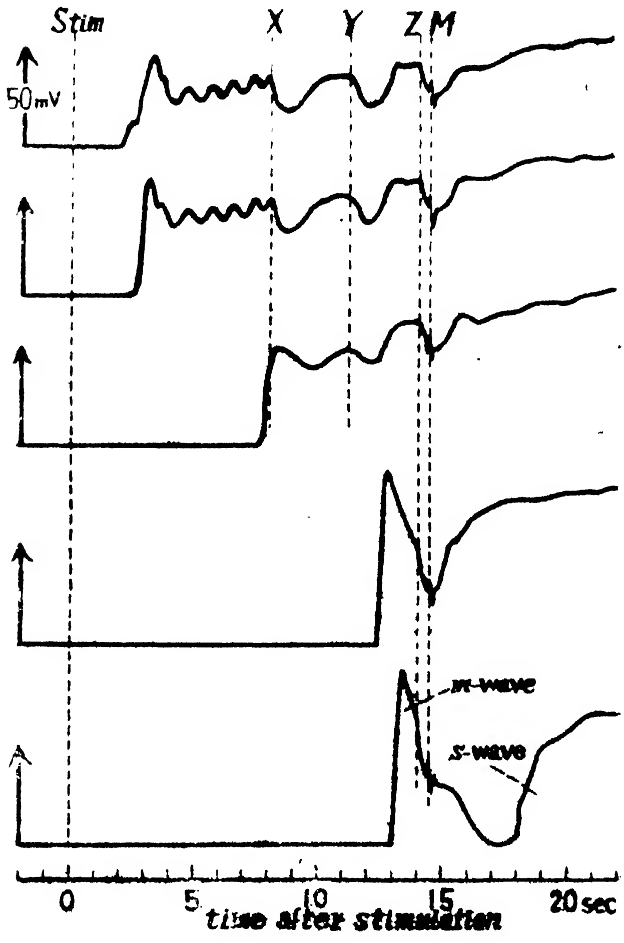

Fig. 2-A

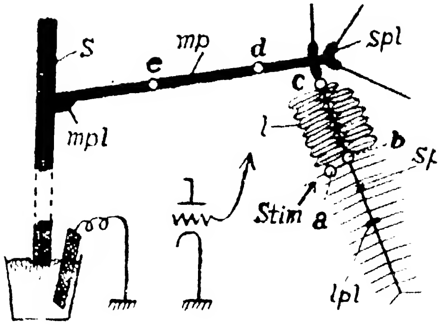

Fig. 2-B

Fig. 2 A. Diagrammatic representations of the action potential curves led off on various points of the leaf of *Mimosa pudica*. Arrangement of each electrodes are shown in Fig. 2 B. *Stim*: Time of stimulation by heating on *Stim* in Fig. 2-B. *X*: *m*-wave reaches to sub-pulvinus. *Y*: *s* wave reaches to sub pulvinus. *Z*: *m* wave reaches to main pulvinus. *M*: Occurrence of turgor variation in the main pulvinus (leaf movement).

Fig. 2-B. Schematical diagram of a leaf of *Mimosa pudica* and arrangement of the leading electrodes (see Fig. 2 A). *s*: stem. *mpl*: main pulvinus. *mp*: main petiole. *spl*: sub pulvinus *sp*: sub-petiole. *lpl*: laminar pulvinus. *l*: leaflet. *Stim*: The point to be stimulated by heating.

are of three kinds: (1) a platinum-iridium needle (0.15 mm. dia.) inserted into the tissue, (2) a fine silver wire coiled around the petiole with additional covering of moistened cotton thread, and (3) a wet cotton thread which connects the petiole with Cu-$CuSO_4$-agar type electrode. No disturbances were caused by the grade of unpolarisability or by different kinds of electrodes on the process of action potential.

The potted plant is shielded from electromagnetic disturbance by covering with a grounding case made of tin-plate and wire gause, and is illuminated by a 200 W lamp. In the majority of experiments one pole of the amplifier connects with a point of the leaf, and the other with a copper plate (5×5 cm.) stuck into the soil of the pot which keeps the potential approximately constant (Fig. 2-B). The leaf of *Mimosa pudica* consists of three parts, a main petiole, one or two pairs of sub-petioles and many leaflets, of which at each basal portion is a motile organ, the so-called pulvinus (Fig. 2-B).

The action potentials to be led off on the main petiole have the simplest pattern and the largest magnitude, as shown in *d* and *e* in Fig. 2-A. They occur in the cases of heating stimulus (to touch slightly with burning joss-stick) applied on the leaflet, sub-petiole, or main petiole. But in the case of cooling (to apply a drop of about 1°C ice water) or electrical (to apply a single shock of open induction current) stimulus, they occur only when it is applied directly on the main petiole. In these cases the action potential does not show a slow variation, here called "*s*-wave", which occur after a quick change of regular form (called "*m*-wave"). The result of cutting stimulus is similar to that of heating, but occasionaly there appears a rapid conduction (60~100 mm./sec., called "*r*-wave"). The action potential accompanying this wave has not yet been proved clearly.

As described above, conductions in the leaf of this plant occur in three types, slow (*s*-wave), moderate (*m*-wave) and rapid (*r*-wave) conduction. The shorter the distance from the leading electrode to the stimulating point, the less the interval between *m*-wave and *s*-wave of action potentials on the main petioles. From this fact it is clear that the rate of conduction of *m*-wave is higher than *s*-wave. From calculation the rate of conduction of the *m*-wave is 20~30 mm./sec., and that of *s*-wave 2~3 mm./sec. (see the next report). The characteristics of excitation in cases either of heating or of cooling and electrical current is as follows: The former produces *m*- and *s*-wave, passing through the pulvinus, while the latters produce *m*-wave only and do not pass through the pulvinus. These facts may be proved by the simultaneous records of action potentials on the basal end of the sub-petiole (*a* in Fig. 3) and on the upper end of the main petiole (*b* in Fig. 3). When the sub-petiole is stimulated by cooling, *m*-wave appears in *a*, but not in *b*, while the stimulation thereafter by heating

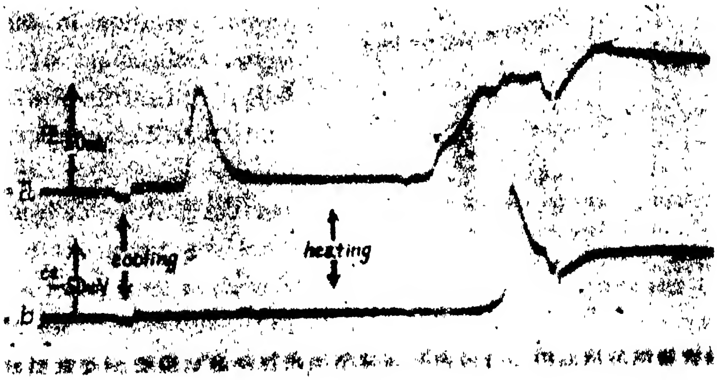

Fig. 3. Curves of action potential led off simultaneously from electrodes *a* and *b* (see the text). Time marks 1 sec. apart.

on the same point produces *s*-wave in both points *a* and *b*. It is noteworthy, however, that some indication of *m*-wave may be found on *s*-wave in *b*, and starts later than *s*-wave. From these results it is clear that *s*-wave can pass only through the sub-pulvinus, producing *m*-wave in the petiole. Therefore, to trace the excitation through the whole leaf the stimulus which can produce *s*-wave must be applied, as already suggested by HOUWINK (1935).

As shown in Fig. 3, *s*-wave has a long duration and an irregular form. HOUWINK called it "the variation", and assumed it to be due to movement of stimulating substance, distinguishing it from "action current". UMRATH considered it as "Aktionsstromgruppen". Now it is proved by the writer, that *s*-wave is distinct from *m*-wave in its mode of conduction.

The *m*-wave in the main petiole has a regular and simple form, and it is similar to the action potential in the nerve, thus it presents an interesting study in physiology of excitation. When the main petiole is directly stimulated by cooling or by electric current at its apical part, it produces basipetal conduction, and action potential (*m*-wave) can be led off on the electrode as in Fig. 4. The duration of rising phase of *m*-waves is 0.3∼0.5 sec., the magnitude −50∼−100 mV. (average: about −80 mV.), and the total duration is 3∼5 sec. The final part of *m*-wave is disturbed by the positive deflection and the rapid biphasic change; the former appears when the excitation reaches to the main pulvinus while the latter corresponds to the occurrence of turgor variation in the main pulvinus (movement of leaf) (HOUWINK, SIBAOKA 1947). If the movement does not occur, the rapid change in *m*-wave never appears, but the positive deflection may be found, so that *m*-wave spreads over the whole length of the main petiole in the usual way (see next report). When the leading electrode is brought near to the pulvinus, the distance between the time of negative deflection of action potential and that of positive one, due to reaction of pulvinus, comes closer (Fig. 5). If the electrode further approaches the pulvinus the positive deflection represses to the magnitude of action potential.

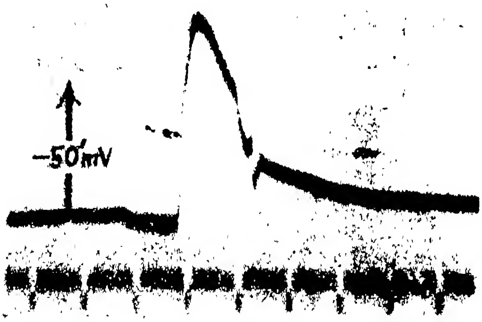

Fig. 4. Action potential accompanying basipetal moderate conduction (*m*-wave) of main petiole. Stimulus direct cooling. Time marks 1 sec. apart.

There are some differences between the action potential led off by stuck electrode and by coiled electrode. In the latter case the magnitude of potential is 30∼40 % larger than the former, and the rising phass is longer. The influence of temperature on the magnitude of potential is usually negligible, with the exception in low temperature (about 17°C.) where the conduction decreases so much that the action potential proceeds slower (longer duration of rising phase). No influences were caused by the differences in the intensity of stimulus and in the distance from stimulating point to leading electrode on the form and the process of action potential (*m*-wave).

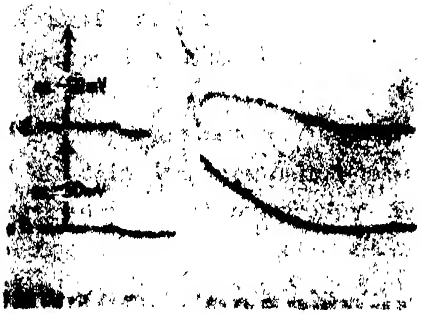

Fig. 5. Two action potentials recorded synchronously on the two points of main petiole. Electrode *a* set on 30 mm. and *b* on 15 mm. above the main pulvinus. The stimulus by direct cooling applied above the leading electrode. Time marks 1 sec. apart

The action potential which accompanies with the acropetal conduction of excitation in the main petiole is shown in Fig. 6 (the earth electrode set on the sub-petiole). Its form and propagation do not differ from that of basipetal conduction. But when the earth electrode is set on either two upper sub-petioles (*II*, *III* in Fig. 6), it shows two positive deflections (Fig. 6, *A*) when the excitation reaches to the sub-pulvinus If the earth electrode is set on the lower sub-petioles (*I*, *IV* in Fig. 6), only one positive deflection appears (Fig. 6, *B*).

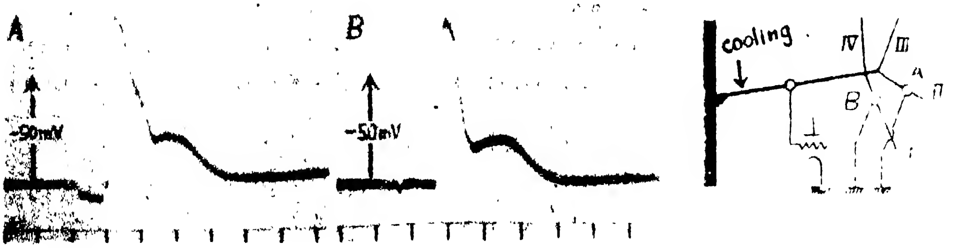

Fig. 6. Difference of numbers of additional positive deflection in acropetal conduction. *A*. The earth electrode set on sub-petiole *II* or *III*. *B*. The earth electrode set on sub-petiole *I* or *IV*. Time marks 1 sec. apart.

Thus on the action potential in the petiole of this plant, the additional positive deflections appear corresponding to the number of pulvinus which exist between the two electrodes, so that we can expect the action potential in the sub-petiole having a complex pattern. The action potentials which led off simultaneously from two points of a sub petiole are shown in Fig. 7. The electrode *a* was set on the apical portion of a sub-petiole, and *b* set on the portion between the most basal pair of leaflet and the sub-pulvinus, covering seven pairs of leaflet between *a* and *b*. The stimulus was applied on the top of this sub-petiole by cooling. As the figure shows, the action potential of electrode *a* has seven small additional deflections of which the first one appears during the rising phase and represses the up-curve of action potential, while the last large one corresponds to the excitation of sub-pulvinus. The action potential of electrode *b* is repressed by positive deflection owing to the excitation of sub-pulvinus which is close to this electrode. If the stimulus is applied by heating or cutting, *s*-wave appears and passes through pulvini, so that action potential in sub-petiole gives some complex form (Fig. 1, *a*, *b* and *c*), which consist of *m*-wave, *s*-wave and positive deflections due to pairs of leaflet and both of sub- and main pulvinus.

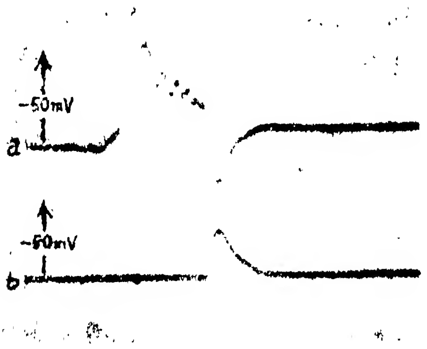

Fig 7. Action potentials led off on two points of sub petiole. See the text. Time marks 1 sec apart

As aforesaid, the positive deflection occur when the excitation reaches to each junction of any parts of leaf (petioles, pulvini and leaflets), where the arrangement of vascular bundles must change itself. Accordingly there deflections may disturb the seeking of the true process of action potential, nevertheless they show the manner of excitatory conduction, and allow us to make use of them for the investigation of the rates of conduction (see the nest report, SIBAOKA 1950).

The writer wishes to express his hearty thanks to Prof. Dr. Y. YAMAGUTI for his supervision throughout this work.

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NATURE OF CONDUCTION OF EXCITATION IN THE PETIOLE OF *MIMOSA PUDICA*.

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(With 4 Text-figures)

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1. *The rate of moderate conduction (m-wave) in the main petiole.*

As previously reported (SIBAOKA (1950)), the action potential in the main petiole which accompanies the moderate conduction of excitation (*m*-wave) has additional positive deflection due to the excitation reaching the pulvinus. From the time difference between the action potential and the positive deflection the rate of conduction may easily be determined.

The rate of conduction, as shown in Tab. 1, closely depends on the maturity of the leaf and of the plant. The young individuals given in tables were less than 20 cm. in height and with about ten leaves. The mature ones were more than 30 cm in height and with about fifteen leaves. Each leaves are numbered

Table 1.

Individual	Leaf number	Temp. °C.	Rate of conduction (mm./sec.)
Young	*III*		28.[illegible]
Mature	*III*		13.1
Young	*I*	[illegible]	40.3
	II	[illegible]	29.1
	IV	[illegible]	21.4
Mature	*II*	2[illegible]	11.2
	IV	2[illegible]	8.[illegible]

from top to base, that is from young to mature. As Tab. 1 shows, the rate of conduction is greater in younger leaves in both cases. The rate of conduction also depends strongly on temperature as shown in Tab. 2; and it may be said that the temperature coefficients (Q_{10}) are related to maturity.

Table 2.

Individual and Leaf No	Temp. (°C.)	Rate of conduction (mm./sec.)	Temperature coefficient (Q_{10})
Young, *I*	29 26	[illegible] [illegible]	3.9
Mature, *I*	29 22	[illegible] [illegible]	3.3
Mature, *II*	30 22	[illegible] [illegible]	2.3

The relation between the rate of conduction and the intensity of stimulus is shown in Fig. 1. The stimulation by a single induction shock was applied on the apical part of the main petiole, the excitation being conducted basipetally. The leading electrode was placed on the middle portion between the stimulating point and the main pulvinus. The rates of conduction are determined in two ways. The first is the measuring of the time interval between the sign of induction shock and the beginning point of action potential (V_1), and the second of the time interval of the beginning point of action potential and the positive deflection (V_2). The intensities of stimuli are expressed by the distance (cm.) between primary and secondary coils of inductorium. When the stimulations are weak, V_1-value is smaller than V_2-value, but when the stimulations are strong, V_1-value is larger than V_2-value, while where stimulations are moderate, V_1 equals V_2. The figure seems to show that V_2-value decreases reciprocally with the increase of intensity of stimulus. This decreasing, however, appears even with no stimulus, during the course of time after the plant was set under the artificial light, so evidently V_2-value does not depend on the intensity of stimulus. There are some indications, on the other hand, that in the neighborhood of the stimulated part the rate of conduction is influenced by intensity of stimulus, while in the distant

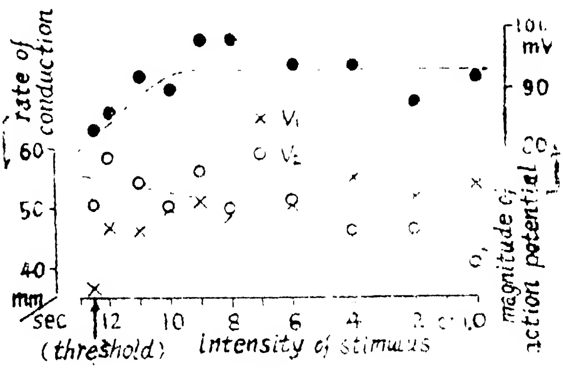

Fig. 1.

parts independency prevails. It is very probable that, in the case of strog stimulation, there occurs "current spread" in the parts neighboring the stimulation. Otherwise the rate of conduction does not depend on the kinds of stimuli ; UMRATH (1937) also came to the same conclusion. The magnitude of action potential is, however, somewhat smaller in the case of weak stimuli, while it does not increase in the case of strong stimuli.

The above mentioned results were obtained by the following experiments Three leading electrodes are set at equal distances between the stimulating electrode at an apical end of the main petiole and the main pulvinus, so that the whole length of the main petiole is divided into four equal sections, numbered from top to base. All leading electrodes are connected with one electrometer, so that the oscillogramm will record the induction shock, three action potentials and a positive deflection. It was formerly confirmed that no effect is evident on the rate of conduction due to short-circuiting of the electrodes, which may allow the measurement of rate of conduction in four sections respectively. Fig.2 shows the result in the case of sections with a length of 12 mm. each. The influence of intensities of stimulus is only confined to the section connected to the stimulating electrode (section *I* in Fig.2), and in other sections the rate of conduction is constant. This behavior may be due to the ground where the weak stimuli may induce slow conduction, but the acceleration of conduction due to the gradual increase in the quantity of excitable units makes the velocity constant, while a strong stimuli may cause the current spread, so that the rate of conduction may appear larger than the true value.

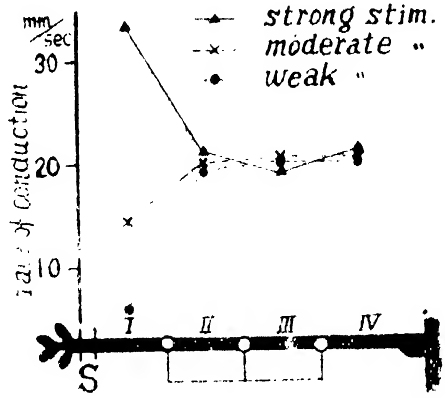

Fig. 2

Table 3.

Individual and Leaf No.	Temp. (°C.	Rate of conduction (mm./sec.)		Acr./Bas.
		Acropetal	Basipetal	
Young, *II*	27 27	31.5 25.8	32.8 25.7	0.96 1.04
Mature, *III*	31 32	11.8 12.9	12.7 12.7	0.93 1.02

All the results above given were for basipetal conductions; similar results, however, were found for acropetal conductions (Tab. 3)

2. *The cutting operation and the transversal spreading of excitation.*

When a small portion of the main petiole is half cut in V-shape in cross, the conduction of excitation is usually interrupted there, reappearing, however, after several hours with a smaller rate of conduction through the connecting intact portion. By the method shown in Fig. 2, the rate of conduction in the half cut portion is estimated and compared with that of the normal portion (Tab. 4). Tab 4 shows that the velocity of conduction decreases only at the half cut portion, with its latter acceleration in the succeeding normal portion.

Table 4.

Hours after cutting	Rate of conduction (mm. sec.)			
	I	*II*	*III**	*IV*
(Before cutting)	9.0	14.[illegible]	1[illegible].	18.3
1	8.[illegible]	12.6	-**	—
2	8.0	14.	11.[illegible]	15.9
3	9.[illegible]	14.[illegible]	12.[illegible]	16.3

* Section with half cut portion

** Conduction interrupted.

In other words, conduction is slowed down on that part where excitable units are fewer than in the normal part, while it is accelerated during the propagation through the portion which has normally more excitable units. These phenomena appear not only in the case of the cutting operation, but also in the case of sticking some needles into the petiole; in the latter case, however, the more numerous the needles, the less the velocity of conduction. The acceleration of conduction seems to be realized by the multiplication of excitable units which is connected with the transversal spread of excitation, as the following experiment shows.

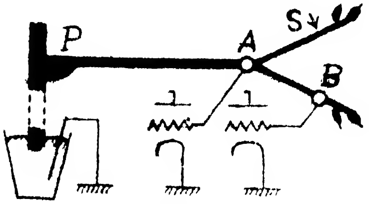

Fig. 3.

The petiole is vertically split into two parts to about 1 cm. below the apical end, one electrode is set on the portion below the two separated parts (*A* in Fig. 3), and the other on the apical end of the split petioles (*B*). Then action potentials of both *A* and *B* are synchronously recorded. When the stimulation is applied on the apical end of the other split (*S* in Fig. 3), we can catch the action potential at B after at A. This fact may be accounted for the ground where the excitaion starts and is conducted basipetally from the stimulated point, while at the base of the two split parts it transversely spreads out to the part which is not yet excited, and begins to anew the acropetal conduction from the electrode B. The rates of conduction from A to the main pulvinus (P) and from A to B, togetner with the magnitudes of action potentials of A and B, are given in Tab. 5. Both the rates of conduction and the magnitudes of action potential in the split part are smaller than in normal parts, which may indicate that the magnitude of action potential is conditioned by the quantity of excitable uuits.

Table 5.

Individual and Leaf No.	Temp. (°C.)	Rate of conduction (mm. sec.)		Magnitude of action potential (mV.)	
		$A - P$	$A - B$	A	B
Young, *I*	30	32.3	11.6	82	43
Young, *I*	30	32.6	28.8	88	51
Young, *II*	34	31.3	25.0	87	46

3. *The moderate conductions in the sub-petiole.*

The rates of moderate conduction in the sub-petiole are smaller than those of the main petiole, being about 9 mm/sec. in the mature leaf at 30°C. The difference in the rates of conduction toward acro- and basipetal directions are not found as in the main petiole, the excitation, however, is not conducted constantly, having a higher conduction velocity in the more basal parts. On the action potential curve in the case of basipetal conduction we can see that (*ref.* Fig. 7 in previous report), the time interval of the small deflections which are caused by pairs of leaflets becomes less and less, while in the case of acropetal conduction the reverse is true. These behaviors may be explained by that owing to the formation of many leaflets on the sub-petiole, the quantity of the excitable units in the latter decreases in the direction from base to top, corresponding to the decrease of size of the sub-petiole in this direction. In the middle portion of the sub-petiole, having 6 ~ 12 constant intervals between pairs of leaflets,

Table 6.

Directions of conduction	Time of conduction (sec.)		
	In whole	In half part	
		Basal	Apical
Acropetal	3.72	1.51	2.21
Basipetal	3.84	1.40	2.44

we can easily confirm the difference in the rates of conduction between the basal half and the apical one, as shown in Tab. 6.

4. *The refractory phase.*

In majority of the experiments in main petiole, the *absolute* refractoty period of moderate conduction is estimated at 150~200 sec., and the relative one at 300~400 sec. The recovering processes in the relative refractory phase at 25°C. are shown in Fig. 4. The magnitude and rising phase of the action potential recover more rapidly than the rate of conduction. This fact seems to suggest that when the conductions of all excitable units reach a uniform level, the magnitude and rising phase of the action potential attains the normal value, so that the plant is not freed from the relative refractory period, until the velocity of excitatory conduction in every unit completely recovers. The absolute refractory period on the stimulated portion, on the other hand, is shorter than that of the other portions, being about 20 sec.

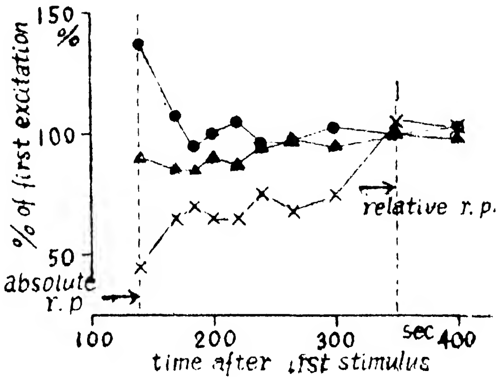

Fig. 4. Recovering curves in the relative refractory phase. ●, rising phase of action potential. ▲, magnitude of action potential. ×, rate of conduction.

5. *Summary.*

(1) In moderate conductions of excitation in the main petiole of *Mimosa pudica*, there is no difference between the conductions toward two directions, acropetal and basipetal (see Tab. 3).

(2) The rate of conduction closely depends on the maturity of leaves (Tab. 1) and on the temperature (Tab. 2); with the exception in cases of neighboring parts to stimulated portions, it is independent on the strength of stimuli (Fig.1).

(3) The excitation propagates decrementlessly in normal state through the whole of the main petiole with a constant velocity (Fig. 2), though in some cases acceleration of conduction may occur (Fig. 2 and Tab. 4).

(4) The velocity of conduction and the magnitude of action potential in one half of a vertical split of the main petiole, are less than the normal value (Tab. 5).

(5) *The excitation spreads transversely at the position connecting two splits* (Tab. 5 and *Fig.* 3).

(6) *It is assumed that, there are several numbers of* excitable units in the petiole which may be appointed in cross section. If the quantity of units is decreased in some way the velocity of conduction and the magnitude of action potenital also decrease. The transversal spreading of excitation from excited unit to the non-excited one, causes the acceleration of conduction.

(7) The recovering process in the relative refractory phase also supports the assumption of the existence of several excitable units

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STUDIEN ÜBER DIE BILDUNG ORGANISCHER SÄUREN IN GRÜNEN PFLANZEN.

III ÜBER DAS VERHÄLTNIS ZWISCHEN DEM SÄUREGEHALT UND DEM N- UND C-UMSATZ VON *BEGONIA EVANSIANA* ANDR.[1]

VON

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(Mit 5 Textfiguren)

(Eingegangen am 31 Aug. 1948)

I. Einleitung

In meiner vorigen Mitteilung zog ich den Tages- und Nachts-N-Gehalt in Betracht in Zusammenhang mit dem Säuregehalt der intakten Blattspreite von *Begonia Evansiana* ANDR., die mittels Wasserkultur gezogen wurden. In dieser Mitteilung beschäftigt es sich nun hauptsächlich mit den Versuchen über das Verhältnis zwischen dem N- und C- einerseits und dem Säuregehalt der genannten Pflanze anderseits, die wegen des Zeit- und Materialsmangel bisher unterblieben sind. Und zwar wir wollen jetzt feststellen:

1. Die Schwankung des Säuregehalts in ein und derselben Blattspreite während des ganzen Tages.
2. Die gegenseitigen Schwankung der Säure und der verschiedenen N-Fraktionen innerhalb der Tages- bzw. Nachtszeiten.
3. Eventuellen Unterschied zwischen den abgeschnittenen und intakten Blattspreiten in der genannten Beziehung
4. Verschiebung der Menge der verschiedenen N-Fraktionen zur Säure bei künstlicher N-Anreicherung.

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[1] Die hier mitgeteilten Ergebnisse beruhen in der Hauptsache auf Versuchen die in den Jahren 1933-1937 ausgefuhrten wurden. Wegen des persönlichen Grundes des Verfassers für den Jahren 1937-1948 wurde die Veröffentlichung stark verzögert.

5. Gegenseitige Schwankung der Säure und der verschiedenen N-Fraktionen beim Kohlenhydratmangel.
6. Das Verhältnis der Schwankungen des Zuckergehalts infolge künstliches Kohlenhydratmangels zur Säuremenge.
7. Den Gehalt der Säure- und der verschiedenen N-Fraktionen bei der männlichen und weiblichen Blüte.
8. Die Atmung in ihrer Beziehung zum C- und N-Stoffwechsel.

II. Versuchsmaterial und Arbeitsmethode.

Als Versuchspflanze diente mir auch hier wie in meiner vorigen Mitteilungen *Begonia Evansiana* Andr., deren Bulbillen am Ende Oktober des vorigen Jahres gesammelt und nach derselben Kulturmethode wie beiden vorigen Versuchen gezogen wurden. In der künstlichen Nährlösung, über die auf die I. Mitteilung zu beziehen ist, gedieh die Versuchspflanze sehr gut und zwar bis zum Ansetzen der Blüten Seit dem Jahre 1934 kam ich im Stande zu sein, immer die Versuchspflanze so gut in Wasserkultur zu ziehen, wie sie 80 cm. hoch erreichte und eine grosse Menge der reifen Samen trug.

A. Bestimmung der Oxalsäure und der N-Fraktionen.

Zur Bestimmung der in wasserlöslichen und wasserunlöslichen Formen existierenden Oxalsäure und der 6 N-Fraktionen wurden dieselben Mikromethode angewandt, die sich in meiner vorigen Untersuchungen als zuverlässig erwiesen hatten und in der betreffenden Mitteilung näherer beschrieben wurden.

B. Bestimmung des Zuckers.

Es gibt zahlreiche Zuckerbestimmungsmethoden, doch jeder Methode haftet zwar die grosse Vor- und Nachteile an. Für meinen Zweck kommt nur eine Mikromethode ins Betracht. Nach langen Vorversuchen fand ich die Hagedorn Jensensche Methode zur Blutzuckerbestimmung auch bei unseren Fall mit Begonien am geeignetesten und zwar mit einigen kleinen Modifikationen für die Analyse der einzelnen Zucker. Diese äusserst empfindliche Methode, die den Zucker als Glukose bis zu 0.02 mg erfassen lässt, bedarf aber einer Übung. Es ist daher dringend zu empfehlen, vor der Bestimmung des Zuckergehalts im Pflanzenmaterial eine Anzahl von Übungsanalysen an reinen Zuckerarten und an beliebigem Material vorzunehmen

a. Allgemeine Bemerkungen.

Nun wollen wir zunächst einige Bemerkungen auf die zur Bestimmung notwendigen Reagenzien, Extraktion Aufbewahren, Reinigung und Hydrolyse des Extraktes zufügen. Der Titer der frisch bereiteten Natriumthiosulfatlösung ist bekanntlich nicht konstant. Einigen Tagen kann der Titer gleich bleiben, aber er wird ganz allmählich immer schwächer. Die Haltbarmachung des Titers von n/200 Natriumthiosulfatlösung durch den 0.2 %igen Natriumfluorid war nicht vollkommen und es liess längstens nur eine Woche lang unverändert, sodass immer eine gelegentliche Kontrollierung nötig war. Dagegen war n/500 Kaliumjodatlösung, die zur Einstellung der n/200 Natriumthiosulfatlösung benutzt wird, lange Zeit haltbar. Um die Kaliumferricyanidlösung, die vom Zucker reduziert wird, vorher alkalisch zu machen, benutzte Hagedorn und Jensen das Natriumkarbonat. Aber in meinen Versuchen benutztes Reagens abweicht von Hagedorn Jensenscher Originalmethode in dem Punkt, dass das Natriumkarbonat beseitigt wurde; abgesondert stellte ich zwar 10%ige Natriumkarbonatlösung her und von denen wurde eine bestimmte Anzahl Tropfen kurz vor der Analyse zum Gesamtgemisch zugegeben. Die Zinksulfat-Natriumchlorid-Jodkaliumlösung sollte nur in kleinen Menge vorrätig gehalten werden; gelegentlich wurde sie trotzdem allmählich gelbbraun gefärbt, aber hat kein Einfluss auf die Zuckerbestimmung, da das abgeschiedene freie Jod durch den Blindversuch mitbestimmt werden kann. Die n/10 NaOH- und 0.4%ige $ZnSO_4$-Lösung wurde jedesmal kurz vor der Anlyse aus Stammlösung (1 nNaOH und 4.5% $ZnSO_4$), die immer vorrätig war, hergestellt. Die lösliche Stärke wurde, wie Lehmann empfohl, nicht in gesättigter Natriumchloridlösung, sondern bloss in destilliertem Wasser gelöst und vor jeder Analyse neu hergestellt. Um die Fehlergrenze, die bei Verwendung kleiner Menge Reagenzien enger verbunden sind, möglichst herabzusetzen, habe ich eine schwache Normallösung als die der Originalschrifte verwandt: statt einer n/200 Kaliumferricyanidlösung stellte ich eine n/500 Lösung und dementsprechend auch eine n/500 Kaliumjodatlösung her. Die Natriumthiosulfatlösung liess ich aber n/200 als solche bleiben, um von Hagedorn und Jensen ausgearbeitete Tabelle benutzen können.

Die zur Titration verwendete Bürette, die von Pregl empfohlen wurde und mit automatischer Füllvorrichtung versehen ist soll eine möglichst spitzenausgezogene Ablaufkapillare besitzen und die Aussenwand von Kapillare mit gereinigtem Paraffin bedeckt sein, um sie vor Benetzung zu schützen und bequem noch etwa 0.01 cc Flüssigkeit abmessen zu können. Etwa an die Bürette angelegte Gummiverbindung muss sich zuvor sogenanntem künstlichem Alterungsprozess unterzogen sein; frischer Gummischlauch ist häufig die Ursache von Fehlern. Die Aussenseite der Flasche für Natriumthiosulfat- und Kaliumferricyanidlösung wurde mit schwarzer Emailfarbe die die Lösung vor Lichtzutritt schützt, bestrichen und jede Flasche war mit Natronkalkröhrchen, das die Lösung vor Kohlensäureabsorption schützt, versehen. Ich nahm die Einstellung der Natriumthiosulfatlösung nach Treadwell in folgender Weise vor: 1 cc einer frisch bereiteten 50%igen Jodkaliumlösung wurde mit 5 cc n/200 Kaliumjodatlösung, sowie mit etwa 50 cc Wasser vermischt. Nach Abpipettieren wurde sie mit 2 cc einer etwa 7.5%igen Salzsäure titriert. Gegen Ende der Titration, die durch Entfärben der Lösung erkannt wird, wurden 2 Tropfen der 1%igen Stärkelösung zugegeben.

Die Bestimmung der Saccharose kann in einer reinen Lösung makromethodisch geschehen, z.B. mittels der Polarisation, des Saccharimeters, durch Kristallisation aus Äthanol, durch Fällen mit Strontiumhydroxyd, oder schliesslich durch Vergären. Diese Methoden sind aber zur Mikrobestimmung im Pflanzenmaterial nicht geeignet. Da aber einerseits die Saccharose selbst keine reduzierende Eigenschaft mehr hat, und andererseits ohne Schwierigkeit in Glukose und Fruktose zerlegt werden kann, ist die Hagedorn-Jensensche Mikromethode zur Blutzuckerbestimmung auch für die Analyse der Saccharose brauchbar. In Bezug auf die Zerlegung der Saccharose gibt es mehrere Verfahren, u. a. die Vergären, Hydrolyse oder Inversion. Gewöhnlich bedient man sich aber der Hydrolyse oder Inversion die durch Erhitzen mit Säure oder durch Einwirkung der Fermente bewirkt wird. Vor und nach der Hydrolyse der Saccharose wird mit obenerwähnten Glukosebestimmungsmethoden der Zuckerwert ermittelt und aus der Differenz die Saccharose errechnet, die im allgemeinen zu 100% wiedergefunden wird. Über die Säurehydrolyse sind in chemischen und botanischen Literaturen die mannigfältigsten Angaben zu finden. Die Art der Säure und ihre Konzentration, die Temperatur und die Dauer der Hydrolyse sind sehr verschieden. Für uns hat sich die von Tollenaar angewandte Methode als die günstigste erwiesen und ich fand, dass die Saccharose in der Lösung oder Pflanzenextrakte bei 70°C innerhalb 5 Minuten vollkommen von 2.5%iger Salzsäure hydrolysiert wurde. Die Bestimmung der Saccharose sowohl in reiner als auch im Gemisch mit Glukose hat die erwarteten guten Ergebnisse geliefert; dieser Zucker wurde also innerhalb der Hydrolysezeit von der Salzsäure in hier angewandten Konzentration nicht angegriffen (Tab. 1 und 2). Bemerkt sei, dass die in dieser und den folgenden Tabellen wiedergegebenen Zuckerwerte aus der Reduktion in die Glukose umgerechnet angegeben sind.

TABELLE 1.

Nr	Saccharose mg	Titriert nach HCl-Hydrolyse mg	%
1.	0.100	0.096	98.00
2.	0.100	0.100	100.00
3.	0.100	0.098	98.00
4.	0.230	0.224	97.39
5.	0.230	0.226	98.26
6.	0.230	0.228	99.13

TABEELE 2.

Nr.	Substanz	Gewicht	Sofort titriert mg	Titriert nach HCl-Hydrolyse mg	%
1.	Glukose dest. Wasser	0.050 mg 2.000 g	0.050	———	100.00
2.	〃	〃 〃	0.049	———	98.00
3.	〃	〃 〃	0.049	———	98.00
4.	Saccharose dest. Wasser	0.130 mg. * 2.000g.	———	0.128	98.46
5.		〃 〃	———	0.126	96.92
6.		〃 〃	———	0.128	98.46
7.	Saccharose Glukose dest. Wasser	0.130 mg. * 0.050 mg. 2.000g.	———	0.175	97.22
8.	〃	〃 〃	———	0.178	98.88
9.	〃	〃 〃	———	0.178	98.88

* Berechnet als Glukose

Die leichte Verarbeitung der Zucker im Atmungsstoffwechsel drängt auch eine rasche Analyse des abgenommenen Materials, sodass das frische Material in gewissen Fällen durch Zuschneiden, durch Mahlen oder durch Reiben unter Sandzusatz zerkleinert werden muss. Die schnelle Verarbeitung ist jedoch bei grösseren Serienuntersuchungen nicht immer leicht zugänglich. Es ist daher das Abtöten der Enzyme im Pflanzenmaterial erforderlich, was durch erhöhte Temperatur oder durch Giftstoffe erreicht werden kann. Besonders in neuerer Zeit haben aber mehrere Forscher das Trocken unterlassen.

da bei Anwendung höherer Temperaturen die Gefahr besteht, dass dadurch einige Pflanzenbestandteile zersetzt und den Einflussen auf die Kohlenhydrate bzw. auf deren Bestimmung gelten gemacht werden. Wenn das Pflanzenmaterial stark sauer wäre, wie in unserem Falle, so ist die Substanz vor der Extrahierung durch Zerreiben mit einem Zusatz von Calziumkarbonat zu neutralisieren oder die Anwendung eines Giftstoffes vorzuziehen. Um einen reinen wässrigen Extrakt herstellen zu können, benutzte ich in den vorliegenden Untersuchungen als Giftstoffe Äther. Der Äther bietet den Vorteil in seinen grossen Verdunstungsvermögen.

Im allgemeinen werden die Zucker durch Äthanol extrahiert, dessen Konzentration und Dauer der Extraktion bei den einzelnen Autoren von einander abweichen. Äthanol hat zwei Vorteile, nämlich die Trennung der löslichen Kohlenhydrate von der Stärke und die Trennung der Zucker von verschiedenen störenden Substanzen. Dieser Vorteil der äthanolischen Extraktion steht aber einem Nachteil gegenüber. Die unentbehrliche Abdestillation des Äthanols bei möglichst niedriger Badtemperatur in Vakuum ist umständlich, zeitraubend und hat meistens Substanzverlust zur Folge. Der Äthanol lässt sich aber ebensogut durch Wasser ersetzen, denn erstens ist die in der Pflanze gebildete Stärke in kaltem Wasser unlöslich und zweitens besitzen wir bei der wässrigen Lösung eine wirksame Reinigungsmöglichkeit durch Adsorption an Kohle. LEHMANN's Arbeit hat bewiesen, dass die Stärke nach dem SCHROEDER-HORN'schen Verfahren (=TOLLENS'schen Verfahren) nicht extrahiert wird, während alle übrigen Zucker restlos gelöst werden Damit ist der wichtigste Vorteil, den die Äthanolextraktion bietet, hinfällig, und überdies wird der Nachteil der zeitraubenden Äthanoldestillation im folgender Weise vor: Das Blatt wird in einen Glasmörser unter geringem Calziumkarbonat- und Quarzsandzusatz zerrieben, mit dem Wasser aufgeschwemmt und dreimal je 5 Minuten lang gekocht. Die in der Eisschrank unter Toluolzusatz aufbewahrte wässrige Pflanzenextrakt ändert erst nach einigen Tagen den Reduktionswert, während die bei der Laboratoriumstemperatur aufbewahrte Extrakt schon am nächsten Tage einen anderen Titrationswert zeigte. Toluol wirkt erst in stärkerer Konzentration reduzierend. so dass diese Reduktion unbeachtet bleiben kann, da Toluol ja nur tropfenweise zu grossen Extraktsmengen zugesetzt wird und seine Löslichkeit im Wasser ausserordentlich gering ist. Soweit es eine quantitativ eindeutige Bestimmung des Zuckers in reiner Lösung anbetrifft, so lag der HAGEDORN-JENSEN'sche Blutzuckerbestimmungsmethode keine Schwierigkeit. Trotzdem kann man nur schwer mit solchen Zuckerbestimmungsmethoden, die nicht genau auf eine nur am Zucker zukommende spezifische Reaktion wie die Reduktionsfähigkeit beruht, das einwandfreie Resultat beim Pflanzenauszuge bekommen, da er ausser Zucker eine Anzahl reduzierender Substanzen enthält. Daher muss hier für die Entfernung oder quantitative Erfassung reduzierender Nichtzucker ein Mittel gesorgt werden. Zur Ausfällung der reduzierenden Nichtzucker bedient man sich verschiedener Metallsalze, wie z.B. Bleiazetat, Blei-, Quecksilber-, Calziumkarbonat oder -nitrat, deren Überschuss mit Schwefelwasserstoff, Natriumkarbonat, -bikarbonat oder -sulfat entfernt werden kann. Erwähnt sei noch die Fällung mit 12%-iger Silico-Wolframsäure, die MOTHES mit gutem Erfolg auf die Untersuchung über Nikotinstoffwechsel der Tabakpflanze angewandt hat. Nach SPOEHR eignet sich vorzüglich die Kohle zur Entfärbung und Klärung der Lösung. Und zwar habe ich auch mit der aktiven Kohle eine starke Verminderung der reduzierenden Substanzen festgestellt. (Tabelle 3).

TABELLE 3.

Nr.	Pflanzenextrakt*	Reinigungsmittel	Gefundene Glukose mg.
1.	2cc.	-	0.095
2.	〃	-	0.093
3.	〃	Aktive Kohle	0.036
4.	〃	〃 〃	0.034
5.	〃	〃 〃	0.038

* Dieser Pflanzenextrakt wurde nach SCHROEDER-HORN'sche Verfahren aus *Begonia*-Blatt, das im Dunkel 4 Tage lang stand und dessen Frischgewicht ca. 1 g. war, hergestellt.

Bei tüchtigen Nachwaschen mit heissem Wasser wird aber der Verlust an Zucker durch die Adsorptionskraft der Kohle höchstens nur auf 1% der angewandten Zuckermenge verringert. (Tabelle 4). Daher wurde die Kohle als ein vorzügliches Entfärbungs- und Reinigungs- und Klärungsmittel in diesen Untersuchungen allgemeinen benutzt. (KONISHIsche aktive Kohle). (vergl. Tabelle 5)

TABELLE 4.

Nr.	Angewandte Glukose mg.	Reinigungsmittel	Gefundene Glukose mg	%
1.	0.075	.	0.075	100.00
2.	0.075	— —	0.074	98.66
3.	0.075	Aktive Kohle	0.075	100.00
4.	0.075	〃 〃	0.074	98.66
5.	0.075	〃 〃	0.075	100.00
6.	0.155		0.154	99.35
7.	0.155	Aktive Kohle	0.154	99.35
8.	0.155	〃 〃	0.153	98.71

TABELLE 5.

Nr.	Pflanzenextrakt *	Zugesetzte Saccharose (berechnet als Glukose) mg	Titriert nach HCl Hydrolyse mg
1.	2cc.		0.087
2.	2cc.	0.050	0.135
3.	2cc.	0.087	0.174

* Dieser Auszug wurde nach SCHROEDER-HORNsche Verfahren aus *Begonia*-Blatt, das im Dunkel 5 Tage lang stand und dessen Frischgewicht ca. 1g. war, hergestellt.

b. Ausführung der Zuckerbestimmung.

Das gewogene Blattspreite wurde mit der Schere fein geschnitten und unter Zusatz von Äther einige Zeit lang stehen lassen. Nach Abtöten der Enzyme durch Äther wurde das frische Material in einem Glasmörser unter geringem Calziumkarbonat- und Quarzsandzusatz gut zerrieben, in 50cc. fassende ERLENMEYERsche Kolben gebracht, und mit dem Wasser aufgeschwemmt. Dieser Blattbrei wurde jedesmal nach 5 Minuten langem Aufkochen dreimal mit Wasser extrahiert, und zwar nach dem ersten und dritten Kochen blieb der Extrakt 1/2 Stunde lang, nach dem zweiten Kochen 12 Stunden lang stehen. Der filtrierte Extrakt wurde vereinigt, zum Marke des 50cc. fassenden Messkolbens genau gebracht und unter Zusatz von Toluol im Eisschrank aufbewahrt. In einen Praparatglas werden 1cc. n/10 NaOH und 5cc. 0.45%iger $ZnSO_4$-lösung gegeben. Ganz genau gemessene 2cc. des Pflanzenextraktes werden in die Zinkhydroxydschwemmung einpipettiert und die Pipette zweimal mit der Mischung ausgespult, ausgeblasen und die Kohle messerspitzenweise auf Mischung unter Umrühren zugesetzt. Beim Blindversuch setzt man anstatt des Pflanzenextraktes 2cc. destilliertes Wasser und sonst bleibt ganz gleich. Um die reduzierende Zuckermenge zu bestimmen eintaucht man das Praparatglas etwa 10cm. tief im Wasser, erhitzt unter Umrühren im siedendem Wasserbad, wobei das Eiweiss flockig ausgefällt und der reduzierende Nichtzucker adsorbiert wird; dann es wird durch einen kleinen Trichter (Durchmesser 30mm) mit feuchtem entfettetem Baumwollfilter in ein Praparatglas (30×90mm.) filtriert und Trichter und Filter mit 2cc. Wasser nachgewaschen. Zum Filtrat werden 5cc. n/500 Kaliumferricyanidlösung und 5 Tropfen 10%iger Natriumkarbonatlösung zugesetzt und ganz genau 15 Minuten in siedendem Wasserbad erhitzt. Nach 3 minutigem Abkühlen im Leitungswasser werden 3cc. Zinksulfat-Natriumchlorid-Jodkaliumlösung und 2cc. 3%iger Essigsäurelösung hinzugegeben und das ausgeschiedene Jod unter Stärkelösungszusatz mit n/200 Natriumthiosulfat aus einer Mikrobürette gegen Ende titriert.

Zur Bestimmung der Saccharosemenge in Flüssigkeit verführ ich wie folgt. In ein Praparatglas werden ganz genau 2cc. Pflanzenextrakt und 3cc. 2.5%iger Salzsäure genommen. Man erhitzt dieses Praparatglas unter Umruhren 5 Minuten in dem auf 70°C gehaltenen Wasserbad, indem das Glas etwa 10cm. tief im Wasser eingetaucht ist. Die Saccharose wird, bei diesem Zustand vollkommen hydrolysiert. Nach der Hydrolyse wird der im Präparatglas abpipettierte Extrakt mit ganz kleinen Stückchen blauen und roten Lackmuspapier versetzt und dann mit 5n Natronlauge unter Umschütteln genau neutralisiert. Es ist ratsam, die Tropfen nicht direkt auf das Lackmuspapier fallen, sondern längs der Wand laufen zu lassen, da der Umschlag sonst schwer zu erkennen ist. Nach der Neutralisation wurde der Zuckerwert mit obenerwähnter Methode ermittelt und die Differenz zwischen der gewonnenen Wert des hydrolysierten und der des nicht hydrolysierten Extraktes ergab die Saccharosemenge. Um den beim Blindversuch ermittelten Natriumthiosulfatverbrauch von dem beim eigentlichen Versuche erhaltenen Wert abzuziehen, müssen beide Werte erst an Hand der Tabelle in Glukose umgerechnet werden. Aus Tabelle erhaltener Wert wird mit 25 multipliziert, weil die zum Versuch gebrauchte Flüssigkeitmenge 2cc. von der Gesamten 50cc. entnommen war. Ein Blindversuch ist stets unerlässlich, da Kaliumferricyanidlösung ihren Titer mit der Zeit verändert. Die Analysen wurden, mit Ausnahme nur einiger Fälle, wenigstens drei- oder viermal wiederholt.

C. Bestimmung der Stärke.

Es gibt eine Anzahl von Stärkebestimmungsmethode, die entweder auf die jodbindende Eigenschaft der Stärke beruhen (z.B. mittels der titrimetrische Bestimmung) oder die Stärke direkt mit chemischen Mitteln ausfallen lassen. Einwandfrei für die Bestimmung kleiner Stärkemengen sind jedoch nur diejenigen Methoden, die auf einer vorangehenden Verzuckerung der Stärke durch Säure oder Fermente beruhen. Das

Verkleistern der Stärke nach den Angaben in Literaturen und meinen Vorversuchen war erst nach einem Erhitzen 45 Minuten lang auf dem kochenden Wasserbade vollkommen. Erst dann wurde der höchste Prozentsatz der Glukose aus hydrolysierter Stärke wiedergefunden. Ferner geht aus meinem Vorversuche hervor, dass der Zusatz von Kohle und Calziumkarbonat keinen Einfluss auf die Hydrolyse ausübt. Die Stärkebestimmung wurde also wie folgt ausgeführt: Die zuckerentfernten Blattrückstände wurden samt mit dem Filtrierpapier in 50cc. fassendes ERLENMEYERsches Kolben, was 20cc. dest. Wasser enthielt, gebracht und auf dem kochenden Wasserbad 45 Minuten lang erhitzt. Der Inhalt des Kolbens wurde heiss extahiert und der Filtrierpapier mehrmals mit heissem Wasser gespült. Nachdem er bis auf Zimmertemperatur abgekühlt ist, wurden 5cc. 14%iger wasserigen Lösung eines reinen Takadiastase-Präparates zugesetzt. Die Fermenthydrolyse wurde auf dem Wasserbad mit einem Zusatz von Toluol bei 37°C und pH 4.9 (Azetatpuffer) 1 1/2 St. lang durchgeführt Nach der vollkommenen Hydrolyse wurde er genau zum Marke des 50cc. fassenden Messkolbens gebracht. Für jede Bestimmung wurden 2cc. von Flüssigkeit benutzt, indem der Stärkegehalt mittels HAGEDORN-JENSENscher Methode als Glukose bestimmt wurde.

III. Tagesschwankungen des Säuregehalts in der einen Blattspreite.

Versuch 1

In der zweiten Mitteilung dieser Serienpublikation sind die Ergebnisse der Studien über die Tagesschwankungen des Säuregehalts in einer Blattspreite vor Sonnenaufgang und nach Sonnenuntergang berichtet worden. Aus dabei gewonnenen Resultate ist geschlossen, dass der Säuregehalt der Blattspreite vor Sonnenaufgang stets grösser als nach Sonnenuntergang ist. Es liegt deshalb nahe, dass die Säure während der Nacht lebhaft gebildet wird, sodass dessen Gehalt vor Sonnenaufgang am grössten ausfällt und dass sie in der Tageszeit, besonders kurz nach der Sonneanufgang eine erhebliche Verminderung erleiden würde. In den genannten Versuchen wurde aber der Säuregehalt bloss zweimal und zwar in einer Abend- und Morgenzeit bestimmt, und die Säureschwankung in den ganzen Tageszeiten war damals dahingestellt geblieben. So ist dieser Versuch ausgeführt worden, um zu sehen, wie die Schwankung des Säuregehalts Tag und Nacht hindurch verläuft.

Als Versuchspflanzen sind Nr. 16, 20 bzw. 42, genommen. Sie waren je 242, 231 bzw. 235 Tage lang in Kultur gewesen, schon ausgeblüht und je eine grosse Menge von reifen Samen betrugen. Von jeden Pflanzen wurden jedesmal die schwarzgrünen und augenscheinlich ganz gesunden Blattspreiten zur Analyse genommen (vgl. Bemerkung der Tabelle 9). Dieser Versuch wurde von 20 Uhr zu 20 Uhr am nächsten Tage durchgeführt, indem die Materialien mit der Zwischenzeit von je 2 Stunden abgenommen und sofort verarbeitet wurden. Der Sonnenaufgang bzw. -untergang an diesem Tage war 6 Uhr 9 Minuten bzw. 16 Uhr 31 Minuten und am nächsten Tage 6 Uhr 10 Minuten bzw. 16 Uhr 30 Minuten.

Tabelle 6.

Tagesschwankungen des Säuregehalts in der einen Blattspreite. (Material in Wasserkultur gezogen).

(a) Die Pflanze Nr. 16 (242tägige Züchtung im Gewächshaus)

Zeit	20.00- 20.05	22.00- 22.05	00.00- 00.05	02.00- 02.05	04.00 04.05	06.00 06.05	08.00- 08.05
Frischgewicht d. Materials mg.	198.762	205.163	237.412	332.726	209.907	204.640	238.427
Gefundene Oxalsäure mg.	1.651	1.644	2.224	2.903	2.065	1.862	2.125
mg. Oxalsäure in 1000g. Frischgew.	8306.4	8013.1	9367.7	8724.9	9837.7	9098.9	7497.5
Zeit	10.00 10.05	12.00 12.05	14.00 14.05	16.00 16.05	18.00 18.05	20.00 20.05	
Frischgewicht d. Materials mg	270.276	319.929	315.286	268.704	372.498	250.188	
Gefundene Oxalsäure mg.	2.592	2.742	2.294	1.993	3.001	2.104	
mg. Oxalsäure in 1000g Frischgew.	9590.2	8570.7	7285.4	7414.1	8056.4	8409.7	

(b) Die Pflanze Nr. 20 (231tägige Züchtung im Gewächshaus)

Zeit	20.05 20.10	22.05 22.10	00.05 00.10	02.05 02.10	04.05 04.10	06.05- 06.10	08.05 08.10
Frischgewicht d. Materials mg.	143.486	153.846	177.676	279.515	202.985	200.827	207.154
Gefundene Oxalsäure mg	1.366	1.310	1.775	2.762	2.445	2.253	2.027
mg. Oxalsäure in 1000g. Frischgew	9250.1	8515.0	9990.2	9881.4	12045.2	11218.6	9784.9
Zeit	10.05 10.10	12.05 12.10	14.05 14.10	16.05 16.10	18.05 18.10	20.05 20.10	
Frischgewicht d. Materials mg.	182.654	271.533	327.731	317.335	280.173	289.861	
Gefundene Oxalsäure mg.	2.095	3.013	2.164	2.197	3.003	2.818	
mg Oxalsäure in 1000g. Frischgew.	11469.8	11096.3	6602.9	6923.3	10718.2	9721.9	

(c) Die Pflanze Nr. 42 (235tägige Züchtung im Gewächshaus)

Zeit	20.10 20.15	22.10- 22.15	00.10 00.15	02.10 02.15	04.10- 04.15	06.10 06.15	08.10 08.15
Frischgewicht d. Materials mg.	231.225	204.929	204.367	279.407	213.638	241.817	291.864
Gefundene Oxalsäure mg.	1.873	1.959	2.316	3.100	2.534	2.596	3.145
mg. Oxalsäure in 1000g. Frischgew.	8100.3	9559.5	11332.6	11094.9	11861.2	10735.7	10806.4
Zeit	10.10- 10.15	12.10 12.15	14.10 14.15	19.10 16.15	18.10- 18.15	20.10 27.15	
Frischgewicht d. Materials mg.	256.814	277.994	318.301	318.714	351.825	283.292	
Gefundene Oxalsäure mg.	2.987	3.026	2.686	3.131	3.697	2.325	
mg. Oxalsäure in 1000g. Frischgew.	11630.9	10885.1	8427.6	9823.9	10508.1	8207.1	

Bemerkung

Nr. d. Pflanzen	Nr. d. Blattspreite von unten	Grösse d. Blattspreite Breite Länge	Zustand der Blattspreite
16	VII	142mm×188mm	Blattspreite war schwarzgrün. Jedes Individuum ist schon ausgeblüht u. trägt die Früchte.
20	V	139mm×200mm	
42	V	140mm×195mm	

8/XI 1934 bewölkt und häufig regnet.
9/XI 1934 hell und im allgemeinen bewölkt

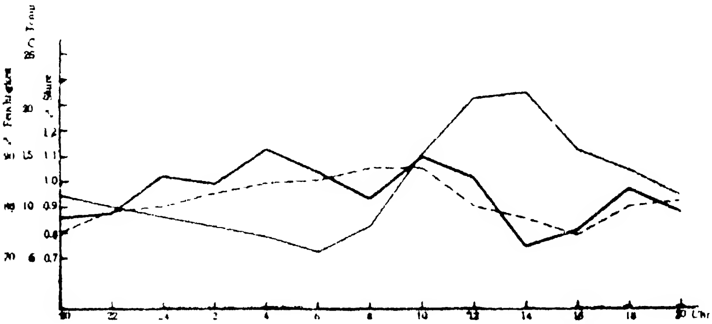

Fig. 1. Tagesschwankungen des Säuregehalts in der einen Blattspreite von *Begonia Evansiana* Andr. (Mittelwerte aus künstlich gezogener Pflanzen)

━━━━ Säuregehalt
- - - - Temperatur
Feuchtigkeit

Die beistehende Tabelle zeigt die Analysenergebnisse. Um diese Verhältnisse anschaulicher zu machen, sind die durchschnittlichen Säureschwankungen mit den im Zeitlaufe entsprechenden Temperatur- und Feuchtigkeitsänderungen nebeneinander auch graphisch dargestellt worden. Aus obiger Tabelle und Figur kann man entnehmen, dass der Säuregehalt der Blattspreite nach Sonnenuntergang allmählich zunimmt und kurz vor Sonnenaufgang zum Maximum erreicht. Nach dem Sonnenaufgang (6–8 Uhr) nimmt der Säuregehalt einen Zeitlang ab, aber steigt mit der Verlauf der Tageszeiten allmählich, und sinkt vor dem Sonnenuntergang (14–16 Uhr) zum Minimum, um wieder allmählich ununterbrochen zu steigen. Es ist somit festgestellt, dass das Maximum des Säuregehalts um 4 Uhr und das Minimum um 14–16 Uhr erreicht wird. In bezug auf die Ursache, die diese Schwankung des Säuregehalts hervorrufen, kann man viele äussere Faktoren, z.B. Temperatur, Feuchtigkeit, Lichtstärke, Nährstoffmenge, CO_2-Gehalt und von der Kombination dieser äusseren Bedingungen abhängige Intensität der Photosynthese, N-Assimilation, Atmung und Transpiration usw. einerseits, und die inneren

Faktoren, die uns noch nicht experimentell zugänglich sind andererseits rechnen. Aber aus dem Vergleichen der Temperatur- und Feuchtigkeitsschwankung mit der Schwankung des Säuregehalts kann man etwa mit Recht sagen, dass die Schwankung des Säuregehalts der Blattspreite im grossen und ganzen umgekehrt proportional zur der Temperatur und proportional zur der Feuchtigkeit ist. Kurz und gut die niedere Temperatur beschleunigt die Säurebildung und *vice versa*. Weiter ist es ersichtlich, dass die Luftfeuchtigkeitskurve mit der Säurekurve beinah parallel lauft. Es gibt dabei nur ein einziges Unterschied, indem die Säurekurve zwar um 8 Uhr eine merkwürdige Senkung gegen die Feuchtigkeitskurve aufweist. Dabei kann die photochemische Zersetzung der Säure, die nicht immer mit den Lebensprozessen der Pflanze gebunden ist, beteiligt sein, wie sie SPOEHR an den verschiedenen organischen Säure bestätigte. Daraufloglende Säurevermehrung kann aber direkt oder mittelbar mit der CO_2-Assimilation bzw. N-Stoffwechsel verbunden sein in dem Sinne, dass die äusseren Bedingungen denn noch stärker die Säurebildung als -abbau vorher zugehen zulassen. Später fällt die Wirkung der äusseren Bedingungen, u.a.der Temperaturerhöhung so aus, dass die Säurebildung trotz der dazu günstigeren inneren Bedingungen so beeinträchtigt wird, dass Säuremenge wegen des photochemischen Abbaues zum Minimum abfallen müsse. Es könnte möglich sein, dass sich der Säuregehalt der Blattspreite unter konstanten äusseren Bedingungen keine nennenswerte Schwankung aufweise. Die gründliche Bearbeitung dieser wichtigsten, Frage mussten aber in Rahmen der vorliegenden Versuchen aus Zeit- und Materialsmangel unterbleiben

IV. Weiteres über den Säure und N Stoffwechsel bei der Blattspreite

1. Versuche an abgeschnittenen Blattspreiten.

A. Von Abend zu Morgen.

a. Freilandpflanzen.

Versuch 2.

Zu diesem Versuch wurden die Blattspreiten verwendet, deren Blattstielen 12 Stunden vor Versuche in den wassergefüllten 50cc. haltigen Kölbchen eingetaucht wurden. Sie waren unter Vermeidung direkter Besonnung und überschüssiger Transpiration der normalen Beleuchtung ausgesetzt. Die Blattspreiten wurden, wie bei meinen Versuchen üblich ist, quer in zwei gleichen Teilen durchgeschnitten. Zur Säure- und N-Bestimmung wurden die eine Blatthälfte am Abend um 22-24 Uhr und die andere am nächsten Morgen um 4-6 Uhr abgenommen und jedesmal sofort verarbeitet. Zur Bestimmung der Säure wurden die Blattstücke von 0.23-0.55g. und zur N-Bestimmung die von 0.39-1.2g. genommen. Der Sonnenuntergang an diesem Tage geschah um 18 Uhr, und der Sonnenaufgang des nächsten Tages um 5

Uhr 10 Minuten. Die Ergebnisse der Analyse sind durchschnittlich in Tabelle 7 zusammengestellt. Ein Blick auf Tabelle 7 zeigt, dass der Säuregehalt der Blattspreiten am Morgen grösser als der am Abend ist. Bezüglich der täglichen N-Schwankungen kann man auch sehen, dass sich die absolute Menge des Gesamt-Eiweiss-, und löslichen N, auf 1000g Frischgewicht des Materials bezogen, am Morgen kleiner als am Abend befindet.

Tabelle 7.
Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite
(Mittelwerte aus 6 Blattspreiten der Freilandpflanzen)
Von Abend zu Morgen.

Bemerkung		Abendbestimmung	Morgenbestimmung
mg Oxalsäure in 1000g. Frischgew.		5762.1	765.2
mg. N in 1000g Frischgew.	Gesamt-N	1975.0	312.8
	Eiweiss-N	1735.2	228.8
	Lösl.-N	239.8	84.0
	Ammon-N	40.6	13.1
	Amid-N	150.1	66.5
	Amino-N	49.1	4.4
Amid/Ammon		3.95	3.89

Der Quotient Amid/Ammon ist bei einzelnen Fällen etwas verschieden, aber liegt meistens niedrig und schwankt 1.76-7.60. Der Mittelwert des Quotienten ist am Morgen etwas kleiner als abends. Aus diesem Ergebnis kann man jedenfalls entnehmen, dass die Abnahme der einzelnen löslichen N-Fraktionen der Säurezunahme am Morgen entspricht. Wenn man in Anlehnung an Desaminierungshypothese über die organischen Säurebildung die Schwankung des Säuregehalts zur des Gehalts an N-Verbindungen gegenüber gestellt hätte, so ist die C-Menge der zugenommenen Oxalsäure viel kleiner ausgefallen, als die aus verlorgegangenen Eiweissmengen zu erwarten ist. Andererseits sind die löslichen N-Fraktionen trotz der stattgefundenen Eiweisspaltung nicht entsprechenderweise zugenommen, sodass der Gesamt-N einen starken Verlust erleiden musste.. Wir haben dabei sicher mit einem Verwundungseffekt zu tun, was eventuelle Beziehung zwischen den oben genannten beiden Stoffwechselprozessen eher maskieren.

b. Künstlich gezogene Pflanzen.

Versuch 3.

Dieser Versuch entspricht dem Versuch 2, ausgenommen die Herkunft von

Material, was im Gewächshaus künstlich gezogen wurde. Nr. 35, 37 bzw. 44 war je 181, 138 bzw. 167 Tage lang in Kultur gewesen und bei Nr. 35 war es schon zur Blüte gekommen. Zur Abendbestimmung wurde das Material um 21–22 Uhr 10 Minuten und zur Morgenbestimmung um 4 Uhr 30 Minuten–5 Uhr 35 Minuten abgeschnitten. Das Frischgewicht des zur Analyse genommenen Blattstückes schwankt 0.11–0.34g. für die Säure, und 0.32–0.54g für die N-Verbindungen. Der Sonnenuntergang an diesem Tage war um 17 Uhr 37 Minüten und der Sonnenaufgang am nächsten Morgen um 5 Uhr 23 Minuten.

Die Ergebnisse der Analyse von Nr. 35, 37 und 44 sind durchschnittlich in Tabelle 8 angegeben. Sie stimmen mit den Ergebnissen des Versuchs 2, sowohl im Säuregeghalt als auch im Gehalt am Gesamt- und Eiweiss-N, der auf 1000g. Frischgewicht des Materials bezogen wurde, gut uberein. Der Gehalt an sämtlichem löslichem N bezogen auf 1000g. Frischgewicht ist dagegen am Morgen etwas grösser als am Abend. Wenn man aber den Mengenverhältnis der einzelnen löslichen Fraktionen der N-Verbindungen in Betracht zieht, so fällt die Summe des Ammon- und Amid-N beinah unverändert aus. Der Quotient Amid/Ammon zeigt ein deutliches Überwiegen des Amids gegenüber dem Ammoniak und schwankt 2.5–6.0.

Tabelle 8.

Tagesschwankungen des Säure- und N Gehalts in der Blattspreite (Mittelwerte aus 3 Blattspreiten künstlich gezogener Pflanzen) Von Abend zu Morgen.

Bemerkung		Abendbestimmung	Morgenbestimmung
mg Oxalsäure in 1000g. Frischgew.		8708.0	1953.0
mg N in 1000g. Frischew.	Gesamt-N	2196.2	72.4
	Eiweiss-N	1914.7	102.3
	lösl.-N	281.5	2[illegible].9
	Ammon-N	36.5	4.9
	Amid-N	189.7	2.0
	Amino-N	55.3	27.0
Amid/Ammon		4.94	4.23

Dieser Versuch ergab wieder, dass die grössere Menge der Säure den 1.kleineren Gesamtmengen des Ammon- und Amid-N entspricht. Wenn aber der Kohlenstoff der verschwindenen Menge des Eiweisses ausschliesslich zur Bildung der Oxalsäure zur Verfügung gestellt worden wäre, so könnte er beinah den Gewinn der Oxalsäure bedecken. Da aber das Gesamt-N einen Verlust erfährt, und die zugenommene Menge der löslichen N-Fraktionen nicht dem Eiweissabbau entsprechend wiedergefunden ist, kann sie der Desaminierungshypothese noch nicht die quantitativ gesicherte Stütze zugeschrieben werden.

2. Versuche an intakten Pflanzen.

a. Freiland Fflanzen.

Versuch 4.

In diesem Versuch wurde Blattspreite von je 3 Pflanzen kurz vor Analyse entnommen in der Weise, dass deren eine Hälfte an bestimmter Zeit abends analysiert, während die andere Hälfte an der Pflanzen belassen und am nächsten Morgen kurz vor dem Sonnenaufgang zur Analyse abgenommen wurde. Zur Bestimmung des Abend-N und der Säure entnahm ich die Blattspreiten um 21-22 Uhr und zu der des Morgen-N. und der -Säure um 4 Uhr 30 Minuten- 5Uhr 25 Minuten. Zur Analyse wurden die Blattstücke von 0.32-0.43g. für die Säure, und 0.82-1.05g. für die N-Verbindungen genommen. Der Sonnenuntergang an diesem Tage war um 17 Uhr 18 Minuten und der Sonnenaufgang am nächsten Tage um 5 Uhr 34 Minuten.

Tabelle 9 zeigt, dass der Säuregehalt der Blattspreite am Morgen grössre als am Abend ist. Diese Ergebnisse stimmen mit denen der Versuche, die mit abgeschnittenen Blattspreiten ausgeführt wurden, gut überein. Der Gesamt- und Eiweiss-N nehmen auf 1000g. Frischgewicht bezogen am Morgen zu während die allen einzelnen löslichen Fraktionen der N-Verbindungen am Morgen abnehmen. Der Quotient Amid/Ammon zeigt ein deutliches Überwiegen des Amides gegenüber dem Ammoniak und ist am Morgen etwas grösser als am Abend. In diesem

Tabelle 9.

Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite (Mittelwerte aus 3 Blattspreiten der Freilandpflanzen)

Von Abend zu Morgen.

Bemerkung		Abendbestimmung	Morgenbestimmung
mg. Oxalsäure in 1000g. Frischgew.		10382.3	+1791.6
mg. N in 1000g. Frischgew.	Gesamt N	2291.8	+ 61.7
	Eiweiss N	2096.2	+105.6
	lösl.-N	195.6	— 43.9
	Ammon-N	35.5	— 14.7
	Amid-N	101.9	— 14.0
	Amino-N	58.2	— 15.3
Amid/Ammon		2.99	4.67

Versuch an intakten Pflanzen unterscheidet es sich also von den vorhergegangenen, dass hier der Einweiss-N am Morgen zugenommen ist, sodass es zeigt, dass die starke Bildung der Säure mit der Eiweisssynthese nebeneinander vor sich gehen kann. Es kann doch undenkbar sein, dass die Säure einzig und allein durch die Desaminierung der Eiweissabbauprodukte entsteht, sodass hier gefundene Eiweisssynthese nur eine sekundäre Erfolge aus der stärkeren synthetischen Tätigkeit darstellt. Meiner Ansicht nach ist aber die Sache nicht so einfach zu beurteilen, weil die Säurebildung, wie man später erfährt, ebenfalls mit der Eiweisssynthese bzw. dem Kohlenhydratabbau in einer ursächlicher Beziehung stehen kann.

b. Kunstlich gezogene pflanzen.

Versuch 5.

Dieser Versuch entspricht dem Versuch 4, ausgenommen des Umstandes, dass hier die Versuchspflanzen in Wasserkultur gezogen wurden. Zu diesem Versuch bestimmte Individuen Nr. 16, 18 bzw. 43 waren je 149, 135 bzw. 135 Tage im Gewächshaus künstlich gezogen und darunter Nr 16 war schon geblüht. Zur Abendbestimmung des N und der Säure entnahm ich von jeder Pflanze die eine Hälfte der Blattspreite um 21-22 Uhr und zur Morgenbestimmung die andere Hälfte der betreffenden Blattspreite um 5-6 Uhr am nächsten Tage. Das Frischgewicht der zur Analyse verwendeten Blattstücke schwankt 0.17-0.31g. für die Säure, und 0.43-0.81g. für die N-Verbindungen. Der Sonnenuntergang an diesem Tage war um 17 Uhr 4 Minuten und der Sonnenaufgang am nächsten Tage um 5 Uhr 42 Minuten. Die Analysenergebnisse sind in Tabelle 10 durchschnittlich zusammengestellt. Daraus geht hevor, dass der Säuregehalt nicht nur am Morgen,

Tabelle 10.

Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite (Mittelwerte aus 3 Blattspreiten künstlich gezogener Pflanzen) Von Abend zu Morgen.

Bemerkung		Abendbestimmung	Morgenbestimmung
mg Oxalsäure in 100g. Frischgew.		1,511.3	+ 3893.4
mg. N in 1000g Frischgew.	Gesamt-N	2229.5	88.0
	Einweiss-N	1928.6	— 36.4
	Lösl.-N	300.9	— 51.6
	Ammon-N	55.0	— 10.1
	Amid-N	162.7	— 27.8
	Amino-N	80.2	— 3.7
Amid/Amino		3.17	2.81

wie immer, grösser als der am Abend ist, sondern die Säurezunahme (3893.4mg. pro 1000g. Frischgewicht) so stark emporstiegen ist, dass wir vorher selten erfahren haben. Die Menge der einzelnen N-Fraktionen bezogen auf 1000g. Frischgewicht des Materials nimmt ausnahmslos am Morgen ab. Der Quotient Amid/Ammon zeigt ein Überwiegen des Amids gegenüber dem Ammoniak, indem er etwa 3 beträgt. Er ist im allgemeinen am Morgen etwas kleiner als am Abend. Was nun das quantitative Verhältnis zwischen den Säure- und N-Stoffwechsel anbetrifft, so ist die Abnahme des Eiweiss-N zu gering, um an dem Kohlenstoff des verschwundenen Eiweissmoleküls die Herkunft des Kohlenstoffes neugebildeter Oxalsäure zu erblicken. Wenn die Desaminierungshypothese für die organische Säurebildung auch hier treffen sollte, so muss man annehmen, dass die gleichzeitig vorgehende Eiweisssynthese den Eiweissverlust grösstenteils bedeckt hatte. Überdies die Abnahme der Eiweissabbauprodukte, u. a. Ammoniak und Amide, ebenso erschwert die Beurteilung, ob man heir als Ursache der Säurevermehrung mit der Desaminierung des Eiwessabbauprodukte zu tun hat oder nicht. Diese Abnahme der N-Verbindung mag bloss ein Ausdruck deren Umwandelung in die anderen Pflanzenteilen sein, sodass die weiteren Versuche zwar an jeden Blattspreiten der betreffenden Pflanzen angestellt werden müssen.

3. Versuche an jeden Blattspreiten intaker Pflanzen.

Versuch 6. (1933).

Um die Tagesschwankungen des N- und Säuregehalts der Blattspreite an intakten Pflanzen der Reihenfolge nach von unten zur Spitze zu bestimmen, nahm ich zur Analyse ein Individum (Nr. 38), das 161 Tage in Wasserkultur gewesen und schon ausgebluht war. Zur Abendbestimmung entnahm ich je eine Hälfte aller Blattspreite um 21-22 Uhr 10 Minuten und zur Morgenbestimmung die eine andere belassene Hälfte um 4 Uhr 30 Min.-6 Uhr. Der Sonnenuntergang an diesem Tage war um 16 Uhr 48 Min. und der Sonnenaufgang am nächsten Tage um 5 Uhr 53 Min. Zur Analyse wurden die Blattstücke von 0.09-0.27g. für die Säure und von 0.32-0.94g. für die N Verbindungen genommen. Hier sei aber bemerkt, dass 7tes Blatt so klein war, dass an ihm nur die Säuregehalt bestimmt werden konnte. Tabelle 11 gibt die hierbei erhaltenen Analysenergebnisse durchschnittlich an. Die Ergebnisse an einzelnen Blättern sind in Fig. 2 graphisch wiedergegeben. An Fig. 2 sieht man zunächet, dass der Säuregehalt in den ganz jungen und alten Blattspreiten geringer als in den mässig gewachsenen ist, und dass die allgemeine Tendenz zur Säurezunahme während der Nacht (Morgenbestimmung) in allen Blattspreiten augenfällig ist.

Durchschnittlich gesehen ist der Gehalt an Gesamt-, Eiweiss- und löslichen N, auf 1000g. Frischgewicht bezogen, im Gegensatz zum durchschnittlichen Gehalt

Tabelle 11.

Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite
(Mittelwerte aus 7 Blattspreiten künstlich gezogener Pflanzen)
Von Abend zu Morgen.

Bemerkung		Abendbestimmung	Morgenbestimmung
mg. Oxalsäure in 1000g. Frischgew.		8270.4	+ 1776.8
mg. N in 1000g. Frischgew.	Gesamt N	2106.8	− 108.6
	Eiweiss N	1775.4	− 95.5
	Lösl. N	331.4	− 13.1
	Ammon-N	62.7	− 4.6
	Amid N	179.9	+ 1.7
	Amino N	89.7	− 10.2
Amid/Ammon		2.94	3.26

an Oxalsäure, am Morgen kleiner als am Abend. Dasselbe ist ebenso beim Gehalt an einzelen löslichen N-Fraktionen, mit der Ausnahme beim Amid-N, der Fall In Bezug auf das Alter der Blattspreite sind der Gesamt-, Eiweiss- und löslichen N neben dessen Fraktionen bei den mässig gewachsenen bzw. der jüngeren Blattspreiten im allgemeinen grösser an Menge als bei den älteren. Der Eiweiss-N nahm während der Nacht am seinen Menge etwa 95.5mg. pro 1000g. Frischgewicht ab. Wenn die entsprechende Menge Eiweiss zur Bildung der Oxalsäure den Kohlenstoff angegeben hätte, wurde die daraus berechnete Säuremenge ganau mit der hier gefundene Menge der Säurezunahme übereinstimmen. Die Bildung der Säure durch Desaminierung der Eiweissabbauprodukte scheint hier eine wichtige Stütze gefunden zu haben.. Trotzdem sind die löslichen N Fraktionen in entsprechender Menge des verschwundenen Eiweisses nicht wiedergefunden, indem sie ebenfalls in der betreffenden Blattspreite losgegangen sind. Und zwar bei alten Blattspreite nimmt der Ammon-N am Morgen ab, dagegen bei jungen Blattspreite zu. Der Amid-N zeigt eine allgemeine Neigung zur Abnahme nach der Reihenfolge der Knoten von der Basis nach der Spitze zu und zwischen den entsprechenden Abend- und Morgenwerten, durchschnittlich gesehen, gibt wenig Unterschied. Bemerkenswert ist es, dass der Amid-N, während der Nacht, von den älteren zu den jüngeren Blattspreiten transportiert wird. Betreff des Amino-N finden sich auch die ähnlichen Verhältnisse zwischen den Abend- und Morgenwerten, wenn auch sie etwa untergeordnet sind. Der Quotient Amid/Ammon zeigt ein deutliches Überwiegen des Amids gegenüber dem Ammoniak ; einzelnen Blattspreiten fällt er verschieden aus ; doch durchschnittlich ist

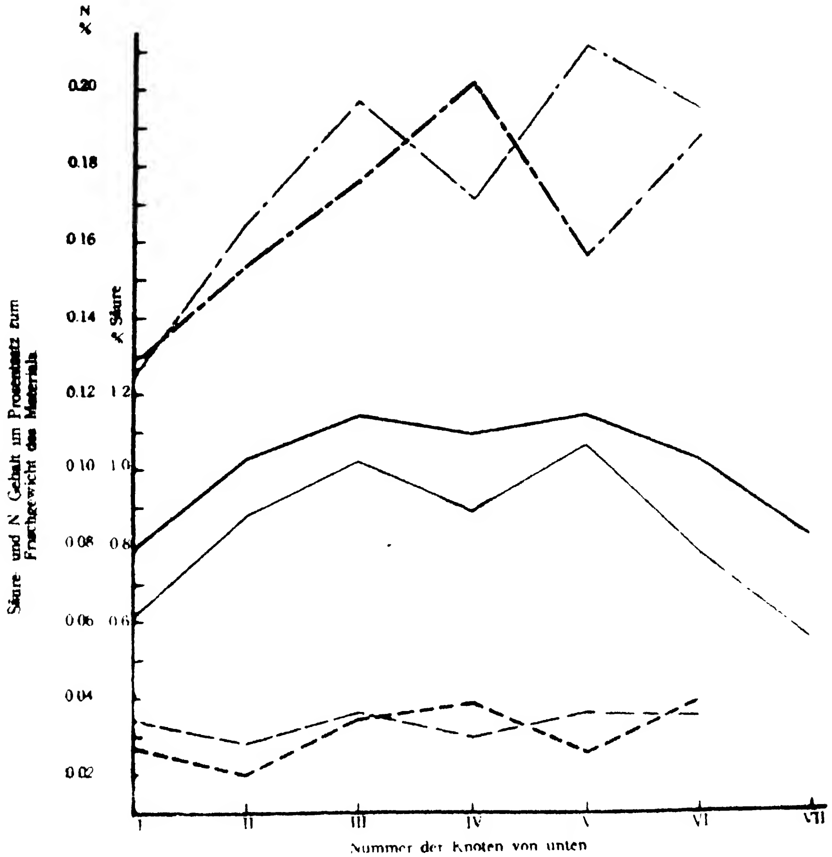

Fig. 2. Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite von *Begonia Evansiana* Andr. (Nr 38, das 161 tägige Kultur in Gewächshaus)

Morgenwerte	Abendwerte
——— Säure	——— Säure
— — — Eiweiss-N	- - - - Eiweiss-N
— — — — Lösl.-N	— — — Lösl.-N

er am Morgen etwas grösser als am Abend, indem er 2.21–4.50 am Abend und 2.44–4.00 am Morgen variiert. Wie man oben gesehen hat, sind die Ergebnisse dieses Versuches so ausgefallen, dass die Säurebildung mit der Desaminierungsprozesse der Eiweissabbauprodukten in inniger Beziehung zu stehen scheint. Der Verlust an den löslichen N-Fraktionen am Morgenbestimmung beruht grosser Wahrscheinlichkeit nach auf deren Fortreisen zu den jüngeren Organen, wie man bei intakten Pflanzen nichts anderes erwarten kann.

Versuch 7. (1934).

Um die im vorgegangenen Jahre gewonnenen Ergebnisse (vgl Versuch 6) weiter nachzuprüfen, wurde an diesem Jahre auch einer gleichgerichteter Versuch durchgeführt. Als Versuchsmaterial wurde die Pflanze Nr. 43 genommen, die 241 Tage lang im Gewächshaus künstlich gezogen wurde. Bei ihr waren die 1. und 2. Blätter schon blassgrün, während die übrigen Blätter noch grün und gesund waren Zur Abendbestimmung wurde das Versuchsmaterial um 20–21 Uhr 50 Min. und zur Morgenbestimmung um 4–5 Uhr 10 Min. abgeschnitten und ohne Aufschub verarbeitet. Der Sonnenuntergang an diesem Tage war um 16 Uhr 28 Min. und der Sonnenaufgang am nächsten Tage um 6 Uhr 15 Minuten. Zur Analyse wurden die Blattstücke von 0.13-0.32g. für die Säure, und die von 0.38-1.13g. für die N-Verbindungen genommen. Die 1. und 9. Blätter waren aber zu klein, um die Bestimmungen sowohl des Säure- als auch des N-Gehalts nebeneinander durchzuführen, sodass bei ihnen immer nur der Säuregehalt bestimmt wurde. Die Analysenergebnisse sind durchschnittlich in Tabelle 12 zusammengestellt und die Befunde an einzelnen Blättern sind in Fig. 3 graphisch dargestellt.

Tabelle 12.

Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite

(Mittelwerte aus 9 Blattspreiten künstlich gezogener Pflanzen)

Von Abend zu Morgen

Bemerkung		Abendbestimmung	Morgenbestimmung
mg Oxalsäure in 1000g Frischgew.		5218.9	+533.8
mg N in 1000g. Frischgew	Gesamt N	2137.4	−121.4
	Eiweiss N	1872.2	−102.7
	Lösl. N	265.2	− 18.7
	Ammon-N	65.4	+ 5.8
	Amid-N	140.1	− 18.7
	Amino N	59.7	− 5.8
Amid/Ammon		2.39	1.61

Fig. 3 zeigt, dass der Säuregehalt der Blattspreite, ausschliesslich der schon blassgrün gewordenen 1. und 2. Blattspreiten, im Übereinstimmung mit dem des vorgegangenen Jahres, am Morgen grösser als am Abend. Der durchschnittliche Gehalt an Gesamt-N, auf 1000g. Frischgewicht des Materials bezogen, fällt am Morgen etwas geringer als am Abend aus. In Betreffs des durchschnittlichen Gehalts an den einzelnen N-Fraktionen ist ersichtlich, dass der Ammon-N dem Eiweiss-N-Verlust entgegen am Morgen etwas zu, während die Amid- und Amino-N-Menge umgekehrt von einem Verlust erleiden ist. Der Quotient Amid/Ammon zeigt wie immer ein Überwiegen des Amids gegenüber dem Ammoniak und ist er am Morgen kleiner als am Abend. Die einzelnen Daten an jeden Blattspreiten

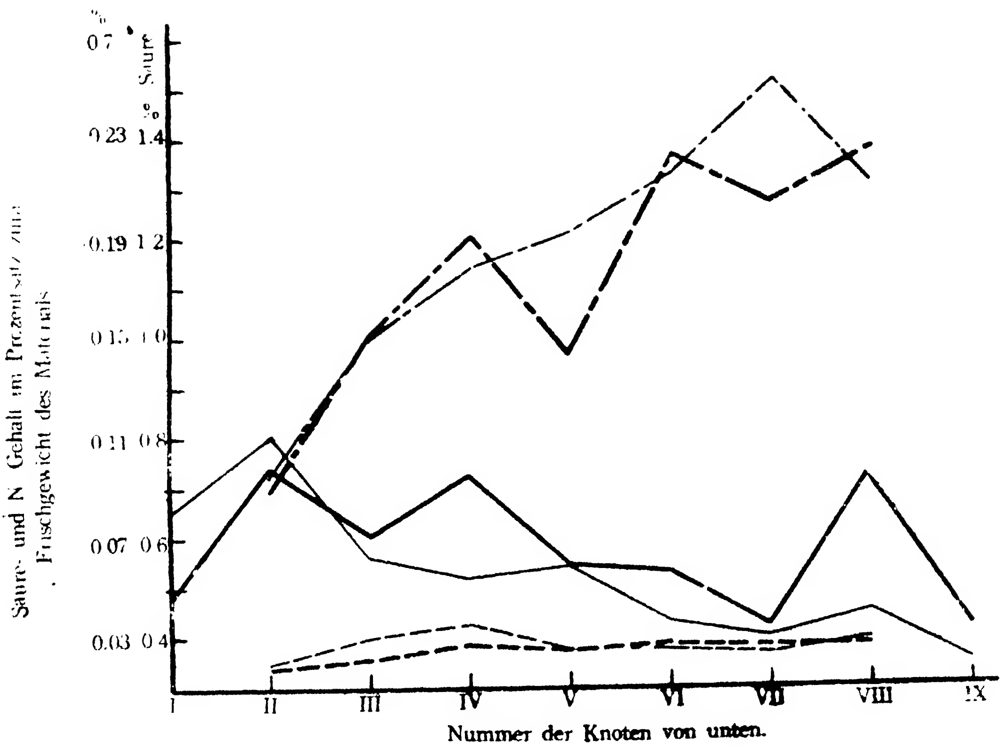

Fig. 3. Tagesschwankungen des Säure und N Gehalts in der Blattspreite von *Begonia Evansiana* ANDR. (Nr. 43, 241 tagige Kultur im Gewächshaus)

Morgenwerte		Abendwerte	
————	Säure	————	Säure
—— — —	Eiweiss-N	- — -	Eiweiss-N
——––––	Lösl.-N		Lösl.-N

(Fig. 3) zeigen uns ein Hinweis dazu, dass zwischen den Schwankungen der Menge des Ammon- (bzw. Amid)-N und der Säure sich eine ursächliche Wechselbeziehung befinden könnte, weil die Kurven beider Schwankungen parallel laufen. Zahlenmässig gesehen ist aber die Zunahme der Menge Ammon-N nicht immer der der Säure begleitet. Andererseits aus durchschnittlichen Angaben in Tabelle 12 ist ersichtlich, dass die C-Zahl der zugenommenen Säure um 1/3 der verlorenen Eiweiss-C ausmacht, sodass in diesem Falle, wenn der Säure-C ausschliesslich von Eiweiss-C abgeleitet werden sollte, die Sache nicht so gut wie beim vorigen Versuche für die Desaminierungshypothese spricht. Ja die Umwandelung bzw. Veränderung der gebildeten Stoffe erschwert die Entscheidung, ob die genannten Stoffe in ihrem Stoffwechsel kausal geknüpft sein sollten oder nicht.

B. Von Morgen zu Abend.

1. Versuche an abgeschnittenen Blattspreiten.

a. Freilandpflanzen.

Versuch 8.

Dieser und nachfolgenden Versuchen unterscheiden sich mit der vorhergehenden zwar in ihrer Durchführung, indem die Analysenreihenfolge in umgekehrter Richtung zu den letzteren ist, sodass die eine Blatthälfte nach 12stündigem Eintauchbleiben in Wasser zuerst am Morgen um 3 Uhr 30 min.-4 Uhr 45 Min. und die zugehörige eine andere Hälfte demnächst am Abend um 22-22 Uhr 56 Min. abgenommen wurde. Er unterscheidet sich also von den vorigen in dem Punkt, dass die Zeitspanne zwischen der ersten und nächsten Abnahme des Blattes grösser bei der vorliegenden als bei der vorigen Versuchsanordnung ist, sodass der Stoffwechsel unter der Tagesbeleuchtung viel weiter oder anders vorangehen kann. Zur Analyse wurden bei 3 Individuen je von den 4. Blatt von unten die Stücke von 0.30-0.39g. für die Säure und die von 0.52-0.93g. für N-Verbindungen genommen. Der Sonnenaufgang bzw. -untergang an diesem Tage war um 5 Uhr 19 Min. bzw. um 17 Uhr 59 Min. Die Analysenergebnisse sind durchschnittlich in Tabelle 13 wiedergegeben. Tabelle 13 zeigt, dass der Säuregehalt ebenfalls am Morgen grösser als am Abend ist. Diese Ergebnisse sprechen ganz gut der Tatsache der täglichen Säureschwankung entgegen, dass der Säuregehalt vor Sonnenaufgang am grössten ist, und, über beträchtlicher Sinkung kurz vor dem Sonnenuntergang, bis zum nächsten Sonnenaufgang allmählich zunimmt. Was den N-Gehalt anbetrifft, so ist die Gesamt- und Eiweiss-N-Menge, auf 1000g. Frischge-

Tabelle 13

Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite

(Mittelwerte aus 3 Blattspreiten der Freilandpflanzen)

Von Morgen zu Abend.

Bemerkung		Morgenbestimmung	Abenbdestimmung
mg. Oxalsäure in 1000g. Frischgew.		6816.9	− 1666.0
mg. N in 1000g. Frischgew.	Gesamt-N	2713.3	− 715.9
	Eiweiss-N	2527.4	− 772.8
	Lösl.-N	185.9	+ 56.9
	Ammon-N	27.8	+ 4.0
	Amid-N	119.3	+ 26.6
	Amino-N	38.8	+ 26.3
Amid/Ammon		4.44	4.59

wicht des Materials bezogen, am Morgen grösser als am Abend, wie beim Säuregehalt der Fall ist. Umgekehrt die einzelnen löslichen N-Fraktionen finden sich am Morgen in kleineren Menge als am Abend. In dieser Hinsicht stimmen die Ergebnisse mit der des vorigen Versuches ganz gut überein, wo die Durchführung der Versuche in umgekehrter Reihenfolge von Abend zu Morgen geschah. Der Quotient Amid/Ammon zeigen ein deutliches Überwiegen des Amids gegenüber dem Ammoniak und schwankt 3.75–5.56 Im Mittel zeigt er aber in seiner Morgen- und Abendbestimmung kein nennenswertes Unterschied Aus Tabelle kann man weiter den augenfälligen Verlust des Gesamt- bzw. Eiweiss-N während der Tageszeiten (Abendbestimmung) entnehmen. Die Zunahme der löslichen N-Fraktionen bei der Abendbestimmung fällt aber dem obengenannten grossen Verlust des Eiweiss-N gegenuber zu klein aus. Überdies die dabei wahrscheinlichen kräftigen Desaminierung der Eiweissabbauprodukte hat augenscheinlich zur Säurezunahme nicht, sondern gerade zur einen von Desaminierungshypothese nicht zu erwartenden Abnahme geführt. Die Säureabnahme am Abend gehöhrt aber zur normalen und regelmässigen Erscheinung bei unserer Pflanze, wie in einem vorigen Abschnitte gesehen haben, sodass wir an der so grossen Abnahme wie 772.8mg des Eiweisses am Abend etwa einen Verwundungseffekt zuschreiben müssten. Jedenfalls zwischen dem Gehalt an Säure und an löslichen N-Fraktionen besteht hier dieselbe Zusammenhang wie beim Versuch 2 und zwar der Säurezunahme am Morgen entspricht die Abnahme der einzelnen löslichen N-Fraktionen. Wenn man aber der Desaminierunghypothese teilnehmen will, würde die Sache so erklärt werden, dass die enorme Abnahme des Eiweiss-N tatsächlich zur Säurezunahme geführt sein sollte, während die Säure wegen der längeren tageszeitlichen Fristen grössenteils photolytisch gespaltet worden wäre. Wie wir aber in vorhergehenden Versuche, wo die Analysenfolge umgekehrt von Abend zu Morgen ausgeführt werde, erfahrt haben, so hätte man ebenso hier auf eines Teilnehmen des Verwundungseffekts Rechnung zu tragen.

b. Künstlich gezogene Pflanzen.

Versuch 9.

Die Kulturdauer der Pflanzen Nr. 10, 13 und 54 war jeder für sich 151, 122 und 95 Tage, darunter war Nr. 10 schon geblüht. Das Entnehmen des Materials für Morgenbestimmung geschah um 3 Uhr 30 Min–5 Uhr 30. Min. und für Abendbestimmung um 21–22 Uhr. Zur Analyse wurden die Blattstücke von 0.11–0.29g für die Säure und von 0.40–0.81g für N-Verbindungen verwendet. Der Sonnenaufgang bzw. -untergang an diesem Tage um 5 Uhr 29 Min. bzw. 17 Uhr 26 Min. Die Analysenergebnisse sind in Tabelle 14 angegeben. Aus diesen Zahlen kann man erkennen, dass der Säuregehalt der Blattspreite wie immer am

Morgen etwas grösser als am Abend ist. In Anbetreff der absoluten N-Menge, auf 1000g Frischgewicht der Blattspreiten, kann man sehen, dass sich die Menge des Gesamt- und Eiweiss-N am Morgen weniger und die der löslichen, u. a. der Ammon und Amid-N-Fraktionen mehr als am Abend findet, was zur Ergebnissen des vorigen Versuches widerspricht. In derselben Beziehung stand der N-Gehalt der einzelnen löslichen N Verbindungen sowohl auf das Gesamt-N als auf das gesamte lösliche N bezogen, obwohl die Daten nicht hier angegeben sind. Der Quotient Amid/Ammon ist in einzelnen Fällen am Morgen meistens kleiner als am Abend, sodass morgens relative geringere Abnahme des Amid-N oder sogar eine Zunahme des Ammon-N geschlossen werden muss, obwohl er im Durchschnitt fast kein Unterschied zwischen den Morgen- und Abendbestimmungen aufweist. Aus diesen Ergebnissen kann man entnehmen, dass beim gegebenen Falle die Säurezunahme am Morgen mit der Zunahme des löslichen bzw. des Ammon- und Amid N im Zusammenhange gehe. Andererseits erwies sich der Eiweiss-N durchschnittlich eine Zunahme beim Abendwerte, was die Eiweisssynthese während der Tageszeiten andeutet. Der an der Eiweisssynthese beteiligte Kohlenstoff würde aber der Wahrscheinlichkeit nach von den Kohlenhydraten zur Verfügung gestellt werden. Wenn die Oxalsäure während der Umwandlung der Kohlenhydrate entstehen könnte, so würde die obengenannte geringere Abnahme der Säure am Abend bei diesem Falle als die beim vorigen durch diese Neubildung der Säure Hand in Hand mit der Eiweisssynthese herbeigeführt sein. Wenn es zutreffen sollte, so könnte die Säurebildung nicht mit dem Eiweissabbauprozesse, sondern gerade mit der Eiweisssynthese mittelbar in inniger Beziehung stehen.

Tabelle 14

Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite

(Mittelwerte aus 3 Blattspreiten künstlich gezogener Pflanzen)

Von Morgen zu Abend.

Bemerkung		Morgenbestimmung	Abendbestimmung
mg. Oxalsäure in 1000g Frischgew.		10674.1	— 417.5
mg. N in 1000g. Frischgew.	Gesamt N	2053.0	+ 61.2
	Eiweiss-N	1715.6	+ 83.9
	Lösl. N	357.4	— 22.7
	Ammon N	74.5	— 18.7
	Amid N	142.8	— 34.9
	Amino-N	120.1	+ 30.9
Amid Ammon		2.68	2.79

2. Versuche an intakten Blattspreiten.

a. Freilandpflanzen.

Versuch 10.

In diesem Versuch diente mir dasselbe Individuum, welches im Versuch 4 benutzt wurde, nur besteht hier der Unterschied von den letzteren in Bezug auf die Lage des Blattes (5. Blatt) und die Reihenfolge der Analysenzeit, indem die eine Hälfte zuerst am Morgen und die andere Hälfte am Abend abgenommen wurde. Zur Bestimmung der Morgen-Säure und des -N entnahm ich das Versuchsmaterial um 4 Uhr 30 Min -5 Uhr 36 Min und zwar Abendbestimmung um 21-22 Uhr 2 Min. Zur Analyse wurden die Blattstücke von 0.21-0.36g fur die Säure und die von 0.56-0.77g für die N-Verbindungen verwendet. Der Sonnenaufgang bzw -untergang an diesem Tage war um 5 Uhr 39 Min. bzw. 17 Uhr 9 Min. Die Analysenergebnisse sind durchschnittlich in Tabelle 15 angegeben. Aus diesen Zahlen kann man vor allem sehen, dass der Säuregehalt, wie in vorige Versuche der Fall ist, am Morgen viel grösser als am Abend ist. Die Zahlen sowohl des Gesamt-N als auch jeder N-Fraktionen bezogen auf 1000g Frischgewicht der Blattspreiten sind ausnahmslos am Morgen grösser als am Abend ausgefallen Der Quotient

Tabelle 15.

Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite (Freilandpflanzen)

Von Morgen zu Abend.

Bemerkung		Morgenbestimmung	Abendbestimmung
mg. Oxalsäure in 1000g. Frischgew.		11073.7	— 1193.8
mg N in 1000g. Frischgew.	Gesamt N	2869.5	— 364.7
	Eiweiss N	2603.2	— 307.3
	lösl.-N	265.3	— 57.4
	Ammon N	46.0	— 12.1
	Amid N	157.9	— 41.9
	Amino-N	61.4	— 3.4
Amid/Ammon		3.52	3.40

Amid/Ammon ist am Abend etwas kleiner als am Morgen, aber im grossen und ganzen beinah konstant. Also die Menge sowohl der Säure als auch der einzelnen N-Fraktionen wurde während der Tageszeiten von der Verminderung erlitten. Unter anderes deutet die Abnahme der löslichen N-Fraktionen sehr wahrscheinlich ihre Wanderung in den Blattstielen an. Andererseits sollte das ziemlich grosse Mengenverschwinden des Eiweiss-N am Abend eine Zunahme der Säure verursachen, wenn sie in ihrer Entstehung hauptsächlich von der Desaminierung der Eiweissabbauprodukte abhängig wären. Dieser Erwartung entgegen ist hier ziemlich grosse Abnahme der Säure gefunden, sodass die Bildung der Oxalsäure mit dem Eiweissabbauprozesse wenig tun zu haben scheint. In diesem Punkt ergänzt dieses Ergebnis zwar den Befunde des Versuches 4 gegeneinander.

b. Künstlich gezogene Pflanzen.

Versuch 11.

In diesem Versuch genommenen Individuen. Nr. 13, 34 bzw. 60 waren je 172, 136 bzw 130 Tage im Gewächshaus kultiviert worden und dazwischen kam Nr 13 schon auszublühen. Zur Analyse wurden die Blattstücke von 0.13-0.28g. fur die Säure, und die von 0.35-0.65g fur die N-Verbindungen verwendet. Zur Morgenbestimmung wurde das Material um 4 Uhr 30 Min.--5 Uhr 35 Min. und zur Abendbestimmung wegen des starken Regens um 21 -22 Uhr des nächsten Tages abgenommen. Der Sonnenaufgang an diesem Tage war um 5 Uhr 49 Min und der Sonnenuntergang an nächsten Tage um 16 Uhr 56 Min. Die Ergebnisse sind durchschnittlich in Tabelle 16 angegeben. Tabelle 16 zeigt, wie immer, dass der Säuregehalt am Morgen etwas grösser als am Abend ist. Der Gehalt an Gesamt-, Eiweiss-, Ammon- und Amino-N bezogen auf 1000g. Frischgewicht des Materials ist am Morgen grösser als am Abend, während der Amid-N ausnahmsweise an der Abendbestimmung etwas zu genommen ist. Der Quotient Amid/Ammon ist am Abend ausnahmslos grösser als am Morgen. Diese Tatsache beruht auf der relative Abnahme des Ammon-N und Zunahme des Amid-N am Abend. Trotz der ähnlichen Ergebnisse beim diesen Versuche mit den vorigen ist augenfällig,

Tabelle 16

Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite
(Mittelwerte aus 3 Blattspreiten künstlich gezogener Pflanzen)
Von Morgen zu Abend.

Bemerkung		Morgenbestimmung	Abendbestimmung
mg. Oxalsäure in 1000g. Frischgew.		1690.1	-- 260.8
mg. N in 1000g. Frischgew.	Gesamt N	2343.4	− 240.6
	Eiweiss N	2061.2	− 222.4
	Lösl. - N	282.2	− 18.2
	Ammon N	48.2	-- 15.8
	Amid-N	161.3	+ 21.6
	Amino-N	72.7	− 24.0
Amid/Ammon		3.34	5.65

dass der Verlust der Säure erheblich und der des Eiweiss-N mässig unterdrückt wurde, wahrscheinlich dadurch, dass die Abendbestimmung von äusserem Grunde an den Zweimal so lange befristeten Blättern durchgeführt wurde. Also die dazwischen vorkommende Neubildung der Säure bzw. Eiweisssynthese kann daran Schuld haben. Wenn die Sache so zu verstehen wäre, so könnte die Säurebildung nicht mit der der Proteolyse, sondern mit deren Synthese vielmehr in innigerer Beziehung gestellt werden.

3. Versuche an jeden Blattspreiten in intakter Pflanzen

Versuch 12 (1933).

Dieser Versuch wurde mit dem Individuum (Nr. 17), das 186 Tage in Wasserkultur gewesen war, ausgeführt. Zur Morgenbestimmung entnahm ich die Blattspreite um 4 Uhr 40 Min.—5 Uhr 30 Min. und zur Abendbestimmung um 21—22 Uhr. Zur Analyse wurden die Blattstücke von 0.06-0.22g. für die Säure, und die von 0.30-1.04g. für die N-Verbindungen verwendet. Der Sonnenaufgang bzw. -untergang an diesem Tage war um 6 Uhr 3 Min. bzw. 16 Uhr 37 Min. Die durchschnittlichen Ergebnisse sind in Tabelle 17 und die einzlenen Ergebnisse in Fig. 4 wiedergegeben

Tabelle 17.
Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite
(Mittelwerte aus 7 Blattspreite künstlich gezogener Pflanzen)
Von Morgen zu Abend.

Bemerkung		Morgenbestimmung	Abendbestimmung
mg. Oxalsaure in 1000g. Frischgew.		11121.4	— 1925.3
mg N in 1000g Frischgew.	Gesamt-N	2368.3	— 217.0
	Eiweiss N	1979.2	— 110.9
	Lösl.-N	389.1	— 106.1
	Ammon N	64.9	— 29.6
	Amid N	215.4	— 40.9
	Amino-N	108.8	— 35.6
Amid/Ammon		4.38	4.51

Nun Tabelle 17 zeigt, dass der Säuregehalt am Morgen grösser als am Abend ist. Anderdrseits ist an der Fig. 4 ersichtlich, dass der Säuregehalt bei den Blattspreiten in der Reihenfolge der Knoten von der Basis nach der Spitze zu abzunehmen neigt, während der Unterschied zwischen Morgen- und Abendbestimmung bei alten Blätter grösser als bei jüngeren Blattspreiten ausfällt. Was den auf 1000g. Frischgewicht bezogenen absoluten Gehalt an Gesamt-, Eiweiss- und allen löslichen N-Fraktionen anbetrifft, so ist der Morgenwert grösser als der Abendwert. Im Gegensatz zum Säurgeehalt steigt der Gesamt- bzw. Eiweiss-N

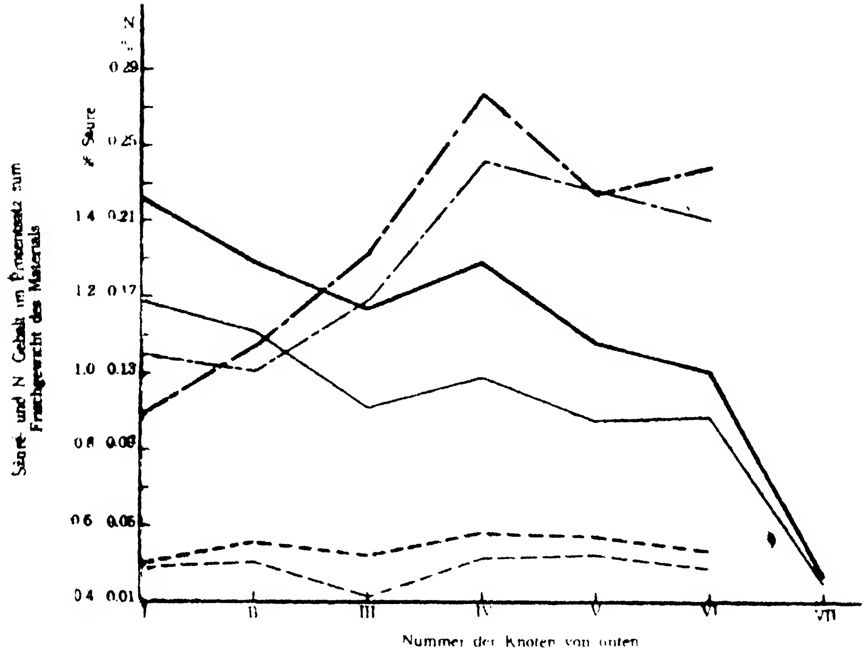

Fig. 4. Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite von *Begonia Evansiana* Andr. (Nr. 17, 185 tägige Kultur im Gewächshaus)

Morgenwerte		Abendwerte	
———————	Säure	———————	Säure
—— — —	Eiweiss N	- - - -	Eiweiss **N**
— — — —	Lösl. N	- · - ·	Lösl. N

allmählich von der Basis nach der Spitze hin auf, während bei den löslichen N-Fraktionen meistens die Neigung zum umgekehrten Verhältnis daran herrscht. Der Quotient Amid/Ammon ist in einzelnen Blattspreiten sehr verschieden, aber zum Amid verschoben; er ist in älteren Blattspreiten am Abend grösser als am Morgen, während die Sache in jüngeren ganz umgekehrt ist. Die Schwankung dieses Quotienten läuft bei der Morgenbestimmung nicht parallel mit der der Säuremenge, obwohl sie bei den Abendbestimmungen eine Neigung zum Parallelgehen miteinander aufweist. Diese Ergebnisse stimmen mit der der entsprechenden intakten Freilandpflanzen ganz gut überein. (Versuch 7). Der tageszeitliche grosse Verlust an der Säure (Abendwert) hat sicher nicht mit dem Stoffwechsel des Eiweisses bzw. mit der Eiweissabbauprozesse zu tun, weil der Eiweiss-N bei den übrig belassenen Blatthälften fast immer von der Verminderung erlitten ist, unabhängig davon, ob man die Analyse in der Reihenfolge von Abend zu Morgen oder der umgekehrten durchgeführt. An der Eiweiss-N-Verminderung in den übrig belassenen Blattälften beteiligt sehr wahrscheinlich der Verwundungseffekt. Also ist hiermit auch nicht sicher nachgewiesen, dass die Oxalsäurebildung mit der Desaminierungsprozesse der Eiweissabbauprodukten in kausaler Beziehung stehen sollte.

Versuch 13. (1934).

Dem vorigen Versuch (1933) entsprechend wurde hier die genannten Ergebnisse noch einmal zur Nachprufung gezogen worden Zur Bestimmung der Tageswerte des Säure- und des N-Gehalts in der Blattspreite wurde ein Individuum (Nr. 50) genommen, das schon 246 Tage lang in Kultur gewesen war. Zur Morgenbestimmung wurde die Blattspreite um 4-5 Uhr 20 Min. und zur Abendbestimmuung um 20-21 Uhr 38 Min. abgenommen und sofort verarbeitet. Die 1., 2. und 3. Blattspreiten waren schon blassgrün geworden, übrige Blattspseiten waren aber noch grun und äusserlich ganz gesund. Zur Analyse wurden die Blattstücke von 0.14-0.35g für die Säure und die von 0.30-1.36g. fur die N-Verbindungen verwendet. Der Sonnenaufgang und untergang an diesem Tage war um 6 Uhr 24 Min. bzw. um 16 Uhr 21 Min. stattgefunden Die Analysenergebnisse sind in üblicher Weise in Form einer Tabelle und Figur wiedergegeben.

Tabelle 18

Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite

(Mittelwerte aus 7 Blattspreite künstlich gezogener Pflanzen)

Von Morgen zu Abend.

Bemerkung		Morgenbestimmung	Abendbestimmung
mg Oxalsäure in 1000g Frischgew		6.799	- 722.7
mg. N in 1000g. Frischgew.	Gesamt N	1993.9	- 133.3
	Eiweiss-N	1734.9	- 125.9
	Lösl.N	259.0	- 7.4
	Ammon-N	79.5	- 0.6
	Amid N	127,9	- 6.6
	Amino N	51.6	- 0.2
Amid/Ammon		1.73	1.58

Es geht aus Tabelle 18 hervor, dass der durchschnittliche Säuregehalt der Blattspreiten am Morgen grösser als am Abend ist. Der Gehalt an Gesamt- und Eiweiss-N. der auf 1000g Frischgewicht des Materials bezogen ist, ist auch am Morgen grösser als am Abend, trotzdem bei den löslichen N-Fraktionen gar kein

Unterschied dazwischen zu erkennen ist. Bezüglich der täglichen N-Schwankungen kann man an der Fig 5 sehen, dass der Gehalt an Eiweiss-N gleichwohl am

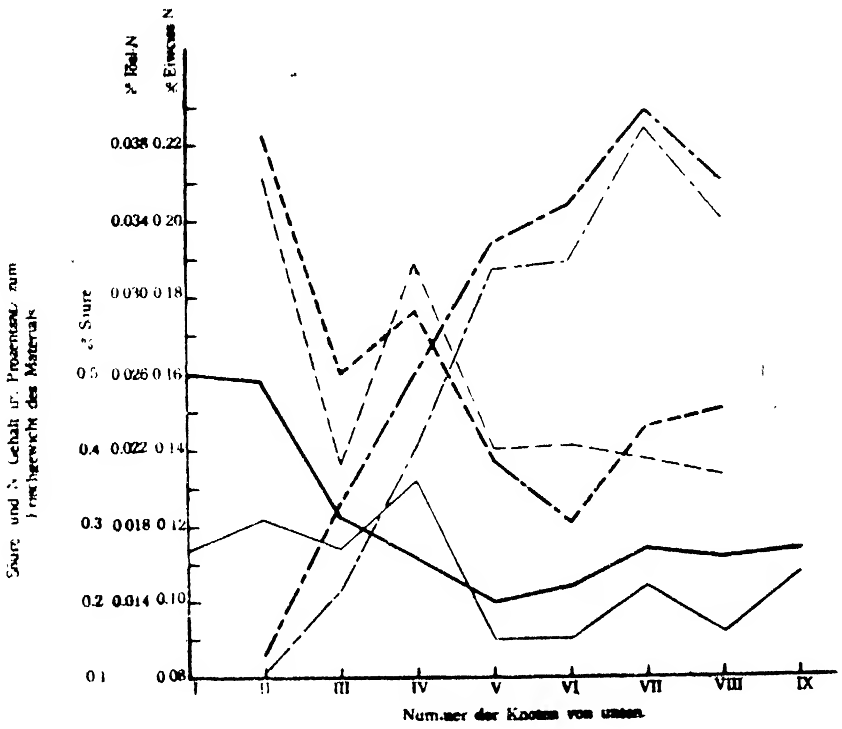

Fig. 5. Tagesschwankungen des Säure- und N-Gehalts in der Blattspreite von *Begonia Evansiana* Andr. (Nr. 50. 246 tägige Kultur im Gewächshaus)

Morgenwerte	Abendwerte
———— Säure	———— Säure
—— — —— Eiweiss N	- - Eiweiss N
— — — — Lösl. N	Lösl. N

Morgen und Abend von den älteren zu den jüngeren Blattspreiten ununterbrochen stark aufsteigt. Dagegen bei den löslichen N-Fraktionen läuft die Reihenfolge meistens in umgekehrter Richtung. Der Quotient Amid/Ammon zeigt ganz schwaches Überwiegen des Amids gegenüber dem Ammoniak und zwischen den einzelnen Ziffern finden sich ganz kleine Schwankungen. Augenfällig ist es auch hier, dass der N-Wert bei den zuerst analysierten Blatthälften immer grösser als dem bei den übrig belassenen ausgefallen ist, unabhängig davon, ob die Versuche von Morgen zum Abend oder umgekehrt ausgeführt werden. Das muss so gedeutet werden, dass die N-Verbindungen, u. a. Eiweiss-N, nach dem Abschneiden der oberen Blatthälfte, in den übrig belassenen durch unbekannte Ursache stark zum Zurücktreten gerichtet wurden, mit anderer Worten, dass wir hier mit dem Verwundungseffekt zu tun haben. Anderseits nimmt die Säure immer während der Nacht zu (Morgenbestimmung), und steht nicht in Beziehung mit der Ordnung der Versuchsausführung (vom Morgen zum Abend oder *vice versa*). Während dieser Versuche ändert sich trotzdem die Kohlenhydratmenge beinah nicht, wie man später in dieser Abhandlung erfahren würde. Dagegen nehmen die N-Verbindungen, u.a. der Eiweiss, immer weiter ab, sodass die N-Menge fast immer in den später ausgeführten Bestimmung kleiner ausfällt. Wenn die Säurebildung mit dem Eiweissabbau in kausaler Beziehung stehen sollte müsste man bei der späteren Bestimmung eine Zunahme des löslichen N. erwarten. Der löslichen N. bzw. Ammon oder Amid-N, vermindert sich der Erwartung entgegen im allgemeinen bei den späteren Bestimmungen, doch in Vergleich zum Eiweiss-N so schwach, dass man ohne Gefahr als unverändert betrachten kann. Alle diese Tatsache sprechen schwer für die Annahme der Oxalsäurebildung durch Desaminierung der Eiweissabbauprodukte in unserer Pflanze.

V. Der Säure- und N Gehalt in der männlichen und weiblichen Blüte.

Versuch 14.

Über das Verhältnis zwischen dem Säure- und N-Gehalt der männlichen Blüten ist schon mitgeteilt worden. (11 Mitteilung. 1933). Um weiter dasselbe Verhältnis in einer und derselben weiblichen und männlichen Blüte zu erforschen, wurden die Analysen durchgeführt, deren Ergebnisse in Tabelle 19 wiedergegeben

Tabelle 19.

Der Säure- und N-Gehalts in den männlichen und weiblichen Blüten.

Pflanzenteil		3 weibl. Blüten, halbentwickelt	4 weibl. Blüten, vollständig-entwickelt	Mittelwert	5 männl. Blüten, vollständig-entwickelt
Frischgew. d. Materials. mg.		446.863	672.931	561.397	320.365
Oxalsäure mg.		2.872	3.225	3.049	2.319
mg. Oxalsäure in 1000g. Frischgew.		6384.1	4792.4	5431.1	7238.6
Frischgew. d. Materials. mg.		621.793	921.624	771.709	439.561
Gesamt-N mg.		0.6380	1.1956	0.9398	0.7658
mg. N. in 1000g. Frischgew.	Gesamt-N	1098.4	1297.2	1197.8	1742.1
	Eiweiss-N	911.4	1114.9	1013.2	1407.7
	Lösl. -N	186.5	182.3	184.4	334.4
	Ammon-N	24.0	38.7	46.4	145.8
	Amid-N	94.6	100.8	97.5	95.5
	Amino N	37.9	43.3	40.6	93.1
Amid/Ammon		1.75	2.59	2.17	0.66

sind. Es geht aus Tabelle 19 hervor, dass der Säuregehalt der männlichen Blüte den der weiblichen Blüte stark überwiegt. Weiter ist daraus zu entnehmen, dass der Säuregehalt der halbentwickelten weiblichen Blüte grösser als der vollständig entwickelten weiblichen Blüte ist. Was zunächst den Gehalt des Gesamt-, Eiweiss- und löslichen N, der auf 1000g Frischgewicht des Materials bezogen ist, betrifft, so ist er bei der männlichen Blüte grösser als bei der weiblichen. Unter den löslichen N-Fraktionen sind der Ammon- und Amino-N bei den männlichen Blüten sogar vielmal so reicher als der bei den weiblichen gefunden. Bei den weiblichen Blüten ist er bei den halbentwickelten etwas kleiner als bei vollständig entwickelten, während der löslichen N-Gehalt bei beiden Entwicklungsstadien ganz ähnlich ausgefallen ist. Der Quotient Amid/Ammon ist bei vollständig entwickelter männlicher Blüte 0.66 im Gegensatz zur vollständig entwickelten weiblichen Blüte 2.59 und halbentwickelten 1.75. Somit es könnte uns veranlassen darauf zu schliessen, dass für die Säurebildung bei den Blüten der N-Umsatz eine hervorragende Rolle spielen soll, weil zwischen dem Säuregehalt und N-Umsatz eine Parallelismus besteht Was man aber anderseits weiter in Betracht ziehen soll, muss da sein, dass sich bei den Blüten gewöhnlich die Atmung so lebhaft vorzieht, dass die Beteiligung des Kohlenhydratumsatzes an der Säurebildung nicht ausgeschlossen werden kann. Um dieser Frage zu entscheiden reicht dieser Untersuchung noch nicht; doch scheint es mir, soweit es das Verhältnis zwischen der Säurebildung und dem N-Umsatz anbetrifft, sehr wahrscheinlich, dass wenigstens bei den Blüten die genannten beiden Vorgänge zueinander innig zusammedhängen, in dem Sinne, dass die von Kohlenhydratumsatz gebildeten Säuren zur Eiweisssynthese eingeführt werden können.

VI. Das Verhältnis zwischen dem Säure und N-Gehalt unter künstlicher N Anreicherung (N-Ernährungsversuche).

Aus den Ergebnisse voriger Abschnitte ist es fraglich geworden, ob die Säurebildung im *Begonia* hauptsächlich auf die Eiweissabbau- oder -aufbauprozzesse abhängig sein kann. Notwendige Folge davon ist es, dass man durch künstliche Regulierung des N-Gehalts in den betreffenden Pflanzenteilen eine weitgehende Einsicht in die Beziehung zwischen den beiden Erscheinungen zu gewinnen strebt. Eine solche künstliche N-Regulierung kann durch Zufuhr der leicht permeierenden N-Verbindungen erreicht werden. Die stickstoffangebotene Pflanze nimmt im allgemeinen sehr energisch den N auf, was selbstverständlich zu einer erheblichen Anhäufung von löslichem N in der Pflanzenzelle führt. Dieser lösliche N geht, nach Untersuchungen mehrerer Autoren, stets zum Ammoniak über. Es ist also der Eigentümlichkeit des Versuchsmaterials überlassen, in welcher Form dieser

Ammoniak entgiftet und deponiert wird, ob als Ammoniumsalz oder irgend einer anderen Form; denn irgendwie muss bei jeder Pflanze die Ammoniaktension auf ein Minimum herabgesetzt werden, anderenfalls sie geht bekanntlich sehr rasch von Ammoniakvergiftung zugrund. Bei der Durchführung dieser N-Ernährungsversuche wurde zum Teil der Methodik M. KULTZSCHER's nachgegangen: Als Nährstoff dienenden Chemikalien stammten grossenteils von KAHLBAUM " Zur Analyse " Reagenzien und wurden erst nach wiedrholter Umkrystallisierung benutzt. Die Versuche mit Ausnahme der Ammoniumsulfat und Calziumnitratfütterungen, wurden steril durchgeführt. Alle N-Lösungen ausschliesslich des Harnstoffs wurden im KOCHschen Dampftopf sterillisiert. Wegen der Zersetzbarkeit des Harnstoffs bei der Hitzsterillisation wurde die Harnstofflösung aber kalt sterillisiert. Der trockene Harnstoff wurde in einem vorher getrockneten sterillisierten Kolben 50 St. lang unter Zusatz von Äthyäther gebracht, dieser dann bei Laboratoriumstemperatur durch Wattebausch abgedampft und sterillisiertes Wasser kalt zugegeben. Fur die Entnahme der verschiedenen N-Lösungen von Vorrat in die Versuchskolben wurden alle mögliche Sorge dafür getragen, um die genannten Lösungen jederzeit steril erhalten können.

Es wurde mit Ausnahme weniger Versuche die abgeschnittenen Blätter der künstlich gezogenen oder Freilandpflanzen benutzt, deren Stiel den Wattepropfen hindurch in die N-Lösung in den 250cc. fassenden Versuchskolben eingetaucht war. Vor dem Einsetzen des Blattstiels in die Versuchskolben, wurde er mit 1% Bromwasser 5 Min. lang sterillisiert und seine Schnittfläche mit Paraffin verschlossen, mit steriles Wasser gewaschen, und danach etwa 2–5cm. oberhalb der Paraffinkuppe abgeschnitten. Die Versuche wurden an zerstreuten Licht im Gewächshaus bei 18–22°C durchgeführt.

A Versuche an abgeschnittenen Blattspreiten

a. Ammoniumsulfatfütterung.

Zum Zweck der N-Anreicherung in der Pflanze wurde zuerst die abgeschnittene Blattspreite in die Ammoniumsulfatlösung eingetaucht; Aus Ammoniumsulfatlösung scheint hauptsächlich das Ammoniumion aufgenommen zu werden, wie OVERTON zuerst feststellte und andere mehrere Autoren bestätigte; daher habe ich in diesem Versuch das Sulfation ausser Acht gelassen. Durch diesen Ernährungsversuch wurde das Gleichgewicht zwischen C und N in der Pflanzen erwartungsgemäss stark zum N hin verschoben. Bloss in dieser Hinsicht gesehen möge dieser Versuch äusserlich ähnlich schien dem Versuche, wo die Kohlensäureassimilation verhindert wurde. Der Unterschied liegt zwar darin, dass bei ben N-Ernährungsversuchen der absoluten Gehalt an Gesamt-N und C gleichsam vergrössert werden kann. Es ist daher interessant zu erforschen, wie sich der Säuregehalt unter diesem Umstande verhält.

Versuch 15.

Zur Untersuchung kamen 3 Pflanzen, die eine im Freiland und die übrigen in Wasserkultur gezogen waren. Unter den letzteren war die eine (Nr. 25) 186 und die andere (Nr. 26) 169 Tage lang in Kultur gewesen und jede stand schon in Blüte. Es wurden von jeder Pflanze zwei Blättern abgenommen, wovon das eine für Kontrollversuch und das andere für Ammonsulfatfütterungsversuch benutzt wurde. Alle Versuchsblätter standen mit dem Stielen 2 Tage lang in der Lösung, die 0.25% Ammonsulfat enthielt; dann wurden sie weiter 1 Tag lang in destilliertem Wasser gebracht, um ein möglichst völliges Entfernen von adsorbiertem und in den Leitungsbahnen angehäuften Ammoniak zu erreichen. Das Kontrollblatt wurde dagegen 3 Tage lang in dem wassergefüllten 250cc fassenden Kolben mit ihrem Blattstiel eingetaucht; destilliertes Wasser wurde je am dritten Tage erneuert. Nach dieser Fütterung waren die Spreiten ganz frischgrün wie vor dem Versuch und äusserlich keine Schädigung festzustellen. Der Versuch wurde den 4. IX. begonnen, den 7. IX. 1935 geendet; die Blätter wurde jedesmal sofort analysiert. Die Analysenergebnisse sind durchschnittlich in Tabelle 20 zusammengestellt.

Tabelle 20.

Der Saure und N Stoffwechsel in abgeschnittene Blattspreiten unter kuntlicher N-Anreicherung

Pflanzenteil		Blattspreite			Blattstiel			Blattspreite + Blattstiel	
Bemerkung		Kontrolle	0.25% $(NH_4)_2SO_4$	0.25% $Ca(NO_3)_2$	Kontrolle	0.25% $(NH_4)_2SO_4$	0.25% $Ca(NO_3)_2$	0.25% $(NH_4)_2SO_4$	0.25% $Ca(NO_3)_2$
mg. Oxalsäure in 1000g. Frischgew.		8159.1	+609.1	+302.7	8973.6	+741.9	+923.0	+675.5	+612.8
mg. N in 1000g. Frischgew.	Gesamt N	1629.0	+554.7	+307.9	596.7	+531.4	+163.5	+543.0	+235.7
	Eiweiss N	1431.5	+398.7	+204.8	385.5	+72.6	+28.0	+235.7	+116.4
	Lösl. N	197.4	+155.8	+103.1	210.2	+458.3	+135.2	+307.3	+119.3
	Ammon N	62.8	+118.2	+101.5	106.7	+380.3	+97.5	+249.2	+89.5
	Amid N	82.5	−7.0	−25.1	78.6	+41.8	+32.2	+17.4	+3.5
	Amino N	52.1	+44.6	+26.7	24.9	+36.7	+25.8	+40.7	+26.3
Amid/Ammon		1.42	0.62	0.48	0.77	0.25	0.65	—	—

Aus Tabelle 20 ist ersichtlich, dass der Gesamt-N-Gehalt im 1000g Frischgewicht des Materials bei den Versuchspflanzen grösser als Kontrollpflanze ist und dabei die deutliche Zunahme des sowohl Eiweiss- als auch löslichen N augenfällig ist. Nach der Ernährung wird der Quotient Amid/Ammon augenfällig kleiner, was auf die stärkere Anhäufung des Ammoniaks beruht. Das Mengenverhältnis des zugenommenen Stickstoffs in verschiedenen Formen ist aber bei den verschiedenen Organen etwas anders ausgefallen. Und zwar bei der Blattspreite hat das Eiweiss-N, im Gegensatz zum Fall des Blattstiels den löslichen N stark überwogen. Das deutet dahin, dass einerseits der Eiweissaufbau im Blattstiele nur eine ganz untergeordente Rolle spielt und dass andererseits sich dort die aufgenommene Ammoniumverbindung in grösseren Menge als solche findet. Trotzdem ist die Zunahme der Säuremenge im Blattspreite der im Blattstiele entgegen klein gefunden. Es ist weiter augenfällig, dass der aufgenommene Ammon N (gefundene Ammon-N und Amid-N gleich 100 gesetzt) im Blattstiel nur bis auf wenigem Prozentsatz in Form von Amid wiedergefunden wurde, während der Amid-N bei der Blattspreite sogar zum Velust geführt wurde. Als ganzes Blatt berechnet beträgt dort aber die in Form von Amid wiedergefundene Ammon-N-Menge nur 22% der aufgenommen, sodass die Eigenschaft der "Ammonpflanze" erhalten bleibt. Zu diesem Sachverhalt in dem N-Stoffwechsel entgegen nimmt für sich der Säuregehalt in den Blattspreiten und -stielen ganz ähnlich zu, sodass die Oxalsäurebildung vielmehr mit der Eiweisssynthese in innige Zusammenhang stehen, und mit dem Eiweissabbauprozesse bzw. darauf folgendem Desaminierungsprozesse wenig zu tun haben kann.

Versuch 16.

Der Versuch 15 leidet von dem Nachteil, dass die Anfangswerte an je einem und demselben Blatt nicht ermittelt werden konnten. Es ist unbedingt notwendig, dass wir wenigstens einmal darüber klar sind, wie der Säure- bzw. N-Stoffwechsel bei einem und demselben Blatt unter der künstlichen Zufügung der N-Verbindungen verändert wird. Zum letzteren Zweck wurde ein Individuum Nr. 28, das für 229 Tage lang im Gewächshaus künstlich gezogen und schon ausgeblüht war, analysiert. Die Blattspreite wurde, wie in meiner ersten Mitteilung dieser Untersuchungsreihe angegeben ist, quer in zwei gleichen Teilen durchgeschnitten und eine Hälfte sofort analysiert. Die eine andere mit dem Blattstiel versehene Hälfte wurde vor Analyse mit dem Stielen zwei Tage lang im 250cc. fassenden, mit 0.25% $(NH_4)_2SO_4$-Lösung beschickten Kolben gebracht und dann noch ein Tag in destilliertem Wasser stehen gelassen. Für den Kontrollversuch wurde die für 220 Tage lang künstlich gezogene Pflanze (Nr. 50) genommen, und die Blätter, in gleicher Weise wie beim N-Ernährungsversuch, anstatt in 0.25%iger $(NH_4)_2SO_4$-Lösung, in destilliertem Wasser eintaucht. Der Versuch wurde den 5. IX,. 1935 begonnen, den 8. IX. geendet und die Blattspreiten wurden ohne Aufschub analysiert. Die erhaltenen durchschnittlichen Ausschläge sind aus Tabelle 21 ersichtlich.

Tabelle 21.

Der Säure- und N-Stoffwechsel in abgeschnittene Blattspreiten unter künstlicher N-Anreicherung

Bemerkung		Kontrolle (H_2O)		0.25% $(NH_4)_2SO_4$		0.25% $Ca(NO_3)_2$	
		Vor Versuch	Nach Versuch	Vor Versuch	Nach Versuch	Vor Versuch	Nach Versuch
mg. Oxalsäure in 1000g. Frischgew.		8319.3	+ 108.7	8126.8	— 1210.5	10504.2	+ 1098.2
mg N in 1000g. Frischgew.	Gesamt N	2225.2	+ 49.3	2968.8	+ 210.6	2837.6	— 30.7
	Eiweiss-N	2071.4	— 8.2	2730.4	— 64.9	2538.7	— 250.3
	Lösl. N	153.8	+ 57.5	238.4	+ 275.5	298.9	+ 169.7
	Ammon N	55.4	— 0.6	42.6	+ 214.3	87.2	+ 92.4
	Amid N	15.5	+ 11.9	113.5	+ 71.5	59.8	+ 134.7
	Amino N	82.9	+ 46.2	82.3	— 10.4	151.9	— 57.5
Amid/Ammon		0.28	0.50	2.66	0.72	0.69	1.08

Die im Wasser gestandene Kontrollpflanze zeigt nach dem Versuch ganz kleine Zunahme an, sowohl der Säure als auch der lösliche und Amino-N. Bei der Versuchspflanze nimmt der lösliche N stark zu, während der Eiweiss-N mässig, aber stärker als bei der Kontrollpflanze abnimmt. Dieser Abnahme des Eiweiss-N, entgegen, ist der N um 2000mg. pro 1000g. Frischgewicht aus Ammoniumsulfatlösung aufgenommen, und um 2/3 davon findet sich in der Form von Ammoniumsalze, sodass sich unter den löslichen N-Fraktionen der Ammon-N enorm und der Amid-N nur mässig zugenommon ist. Was nun den Säuregehalt anbetrifft, so ist er nach der Ammoniumsalzernährung 12mal so gross wie der bei der Kontrolle zugenommen. Wenn der Anteil abgenommenes Eiweiss (64.9mg. × 6.25) gerade zur Säurebildung verarbeitet würde, so müsste die Oxalsäure etwa 1100mg. pro 1000g. Frischgewicht zunehmen, was der gefundene Menge nahe steht.

Die Kohlenhydrate, wie man später in dieser Abhandlung erfahren würde, weisen dazwischen keine nennenswerten Schwankung auf, sodass hier die Eiweissabbauprozesse auf die Säurebildung in inniger Beziehung zu stehen scheint.

b. Calziumnitratfütterung.

Das Nitrat gehört nicht so reichlich verbreiteter N-Form in Pflanzenreich, die Ausnahme davon bilden nur die sogenannte Nitratpflanzen, wie *Amaranthus*, *Chenopodium*, *Urtica*, *Mercurialis*, *Solanum*, *Sinapis*, *Helianthus*, *Capsela* und viel andere. So es bietet ein interessantes Problem das zu erforschen, wie sich die N-Verteilung und der Säuregehalt verhalten, wenn die Begonien in der Nitratlösung gezogen werden.

Versuch 17.

Zum Versuch kamen dieselben 3 Freilandpflanzen, die gerade zum Versuch 15 verwendet wurden. Von jeder Pflanze wurden je 2 Blätter abgenommen, indem das 3. Blatt von unten für den Kontrollversuch und das 4. Blatt zur Nitraternährung benutzt wurde Die abgeschnittenen Blätter der zwei Pflanzen Nr. 25 und 26 standen mit den Stielen 48 St. in 0.25%iger Calziumnitratlösung und darauf noch 24 St. im Wasser; hingegen beim Kontrollversuch standen sie 52 St. in destilliertem Wasser, was nach 48 St. einmal erneuert wurde. Nach dieser Vorbehandlung waren die Blätter sowohl der Kontroll- als auch der Versuchspflanze noch schwarzgrün und äusserlich keine Schädigung daran festzustellen. Die Versuch wurden von den 4 IX. bis 7. IX. 1935 im Gewächshaus ausgeführt und auf der Stelle analysiert Die Analysenergebnisse sind in Tabelle 20 zusammengestellt Aus Tabelle 20 ist ohne weiters ersichtlich, dass bei der Versuchspflanze die ähnlich starke Säurezunahme wie bei Ammoniumsulfatfütterung stattfand Was den Gehalt des N nach der Erhährung anbetrifft, so nimmt der Eiweiss-, und Ammon-N ebenso wie beim Ammoniumsulfatfütterung zu; die betreffende Zunahme beträgt dort aber nur die Hilfe der des letzteren Falles. Aus der Tabelle geht weiter hervor, dass der Anteil aufgenommenes Nitrat-N. (aus denen als Reduktionsprodukt Ammon-N bzw. Amid-N in Betracht kommt) weniger leicht zur Amidsynthese als bei der Ammonium-N verwendet wird. Der Quotient Amid/Ammon zeigt ein deutlicnes Überwiegen des Ammoniaks gegenüber dem Amid und er beträgt bei der Versuchspflanze 0.19–1.27 gegenüber 0.54–1.90. bei der Kontrolle. Jedenfalls sprechen die Ergebnisse vielmehr dafür, dass die Säurebildung mit der Eiweisssynthese, wenn überhaupt der Eiweissumsatz irgendwie damit zu tun hätte, in Beziehung stehen sollte.

Versuch 18.

Um die Ergebnisse des vorstehenden Versuches an einem und demselben Blatt nachzuprüfen wurde ein Individuum Nr. 22, das 229 Tage lang in Gewächshaus künstlich gezogen wurde, zur Calziumnitraternährung verwendet. Es war ca 60cm. hoch, trug 9 Blätter und schon ausblüht. Zum Versuch wurden die an den 4. und 5. Knoten von unten sitzenden Blätter abgeschnitten, deren Blattspreiten quer in zwei gleichen Teilen durchgeschnitten wurden; die einen Hälften würden sofort analysiert, die einen anderen Hälften standen 2 Tage lang in der 0.25% Calziumnitratlösung und dann weiter 1 Tag in destilliertem Wasser mit dem Blattstiel. Die Kontrollpflanze wurde bloss in destilliertem Wasser mit ihrem Blattstiel 3 Tage lang gehalten. Aus der Ergebisse (Tabelle 21) kann man zunächst klar sehen, dass das Eiweiss-N nach der Nitratkulturen stark abnahm, im Gegensatz zum vorstehenden Versuch, wo die mässige Zunahme der Fall war. Andererseits ist es augenfällig, dass die Säurebildung beinah doppelt so gross wie beim vorigen stattfand.

Eine andere Merkwürdigkeit liegt darin, dass hier das Amid-N das Ammon-N überwiegt; beim vorstehenden Versuche war das Verhältnis ganz umgekehrt indem das Amid-N nach wie vor der Nitratkultur fast unverändert blieb. Diese Tatsachen scheinen darauf zu beruhen, dass die ein Teil der hier gefundenen Säuremenge mit der Desaminierung der Eiweissabbauprodukte genetisch gekoppelt entstanden ist, weil hier vermehrte Amid-N-Menge sicher an dem Eiweissabbauprozesse zu verdanken sei. Oder die Sache wäre so, dass die Säurevermehrung beim Blatthälftenversuche von innerer Gründe, etwa sich an der Verwundung geknüpft, anders als beim Ganzblattversuche verläuft.

c. Asparaginfütterung.

Unter den älteren Arbeiten der künstlichen Asparaginernährung bei Blättern sei nur die Arbeit von SAPOSCHNIKOW zu erwähnen, die bei *Vitis*-Blättern auf Kosten von Asparagin die Eiweisssynthese neben einer Vermehrung des Nichteiweiss-N (löslichen N) feststellen konnte. Im Jahre 1926 studierte MOTHES den N-Stoffwechsel von *Vicia faba* und *Phaseolus multiflorus*, unter Asparaginernährung und stellte dabei fest, dass bei einigen Versuchen im allgemeinen die Eiweisssynthese auf Kosten des zugeführten Aspararagins beobachtet werden konnte, während bei anderen diese Erscheinung, trotz ausgiebiger Kohlenhydratzufuhr, nicht zu finden war; Eiweissynthese wird in jungen Blättern bei Gegenwart von genügenden Kohlenhydraten immer hervorgerufen, wenn Asparagin geboten wird; dagegen bei alten Blättern wird ihrer Eiweisshaushalt eben bei Kohlenhydratzufuhr nur schwer oder überhaupt nicht balanziert. Sie bauen Eiweiss ab, wenn auch im Lichtversuch Kohlenhydrat angereichert, oder im Dunkelversuch künstlich Glukose zugeführt wird. Das Blattalter übt grossen Einfluss auf Eiweisssynthese oder Eiweissabbau aus.

Nach Versuchen DIETER's wird die Amidgruppe des Asparagins weder durch gärende noch durch nichtgärende Hefe (sofern sie sich nicht weiter wachsen) abspalten; die gegenteiligen Befunde waren aber von O.v. FÜRTH und FRIEDMANN an autolysierter Brauerhefe und von KURONO an Sakéhefe mitgeteilt worden. Diese scheinbar widersprechenden Ergebnisse der einzelnen Bearbeiter erklären sich zwanglos aus der grossen Empfindlichkeit der Hefeasparaginase, die besonders in wässriger, schwach sauer Lösung zutage tritt. Bei pH 5.0 und 0°C fanden GRASSMANN und MAYR in einzelnen Versuchen schon nach einer halben Stunde mehr wie zwei Drittel des Enzyms zerstört. Die Säureempfindlichkeit dieses Enzyms, der eine relativ höhere Beständigkeit bei alkalischer Reaktion gegenübersteht, ist demnach grösser als die bei irgendeinen der bekannten Hefeenzyme sie übertrifft diejenige der Maltase bei weitem. Nach CLEMENTI liegt aber das

Optimum der Asparaginasewirkung im Gebiet neutraler oder ganz schwach alkalischer Reaktionen. Nach KURONO wirkt Asparaginase aus Bier- und Sakéhefe nicht nur bei schwach alkalischer, sondern auch bei saurer Reaktion. Nach K. SHIBATA und WAKSMAN greifen ebenfalls die Schimmelpilze und vielen Bakterien Asparagin an. Bei höheren Pflanzen fand KIESEL bei *Vicia sativa* und *Lupinus albus* die Asparaginspaltung durch den 4-wöchige Keimpflanzensaft, die nicht durch den Alkohol gefällt worden war. Ganz kürzlich KULTZSCHER wies durch Untersuchungen des N-Stoffwechsel von sogenannter "Amid-" und "Ammonpflanzen" unter Asparaginernährung nach, dass sie unter obenerwähnten Bedingungen ganz verschieden verhalten; während Asparagin in "Amidpflanzen" als spezifische Speicherform liegen bleibt, soweit es nicht zur Eiweisssynthese Verwendung finden kann, wird es in "Ammonpflanze" (Säurepflanze) energisch zu Ammoniak abgebaut, und als Ammonsalz deponiert wird; mit erhöhter Wasserstoffionenkonzentration scheinen auch eine gesteigerte Abbauintensität verbunden zu sein. Jedenfalls bleibt bei höheren Pflanzen erst eine genaue Untersuchungen zu erwarten. Unter diesem Sachverhalt ist es ein interessantes Problem zu erforschen, wie sich die eine sehr saure Pflanze wie ***Begonia Evansiana*** ANDR. gegen Asparaginernährung verhält. Gehen wir jetzt zu unseren Versuchen an.

Versuch 19.

Zur Bestimmung des Säure- und N-Gehalts der Blätter unter Asparaginer nährung wurden die Individuen Nr. 8 bzw, 31, die 180 Tage bzw. 201 Tage lang in Wasserkultur gewesen waren und ein Individuum, das im Freiland gezogen war, genommen. Der Ernährungsversuch wurde in üblicher Weise im Gewächshaus unter Verwendung sterillisierter Lösung ausgeführt. Die Blätter wurden ebenfalls sterillisiert. Der Versuch wurden den 17. IX 1935 begonnen, den 20. IX geendet. Die Analysenergebnisse sind in Tabelle 22 durchschnittlich zusammengestellt.

Aus der Tabelle kommt heraus, dass die Neigung zu Vergrösserung der Säure- und N-Menge unter Asparaginernährung ähnlich, wie bei den vorstehenden Fällen der Kultur mit den anorganischen N-Verbindungen läuft. Die zwischen beiden bestehenden Unterschiede liegen nur darin, dass bei Asparaginernährung neben der zu erwartenden Vorsprung der Amid-N-Menge die doppelt so grosse Vermehrung der Säurebildung wie die bei den Versuchen mit anorganischen N-Verbindungen vorkam. Zur zugenommenen Eiweiss-N-Menge hat sicher der aufgenommene Asparagin-N beitragen. Wenn der zur Eiweisssynthese beteiligte C ebenso von desaminiertem Asparaginreste gestammt hätte, könnte der Asparaginrest keine Beziehung zur Säurebildung haben. Es scheint mir aber sehr wahrscheinlich, dass der zum Eiweissmoleküle gebundene C von den Kohlenhydraten

Tabelle 22.

Der Säure- und N-Stoffwechsel in abgeschnittene Blattspreite unter künstlicher N-Anreicherung

Pflanzenteil		Blattspreite			Blattstiel			Blattspreite + Blattstiel	
Bemerkung		Kontrolle	0.25% Asparagin	0.25% Harnstoff	Kontrolle	0.25% Asparagin	0.25% Harnstoff	0.25% Asparagin	0.22% Harnstoff
mg. Oxalsäure in 1000g. Frischgew.		8169.3	+ 1088.5	+ 1068.1	8770.4	+ 999.1	+ 1130.8	+ 1043.8	+ 1099.4
mg. N in 1000g. Frischgew.	Gesamt-N	2089.5	+ 409.8	+ 784.7	436.2	+ 486.8	+ 636.7	+ 448.3	+ 710.7
	Eiweiss-N	1885.8	+ 275.0	+ 658.0	303.2	+ 13.2	+ 45.1	+ 144.1	+ 351.6
	Lösl.-N	203.7	+ 134.8	+ 126.7	132.7	+ 473.6	+ 591.6	+ 304.2	+ 359.1
	Ammon-N	46.4	+ 112.2	+ 90.5	46.1	+ 185.2	+ 283.5	+ 148.7	+ 187.0
	Amid-N	108.4	+ 26.2	− 8.2	59.3	+ 226.9	+ 228.1	+ 126.6	+ 109.9
	Amino-N	48.9	− 3.6	+ 44.4	27.3	+ 61.5	+ 80.0	+ 28.9	+ 62.2
Amid/Ammon		2.34	0.93	0.81	1.39	1.34	0.73		

herrühre, und dass dabei durch Desaminierung im Überschuss entstandenem Ammoniak entsprechend die Kohlenhydrate auch überschüssig oxydiert werde, sodass es zum Schluss zur Säurezunahme führe. Wenn die hier gefundene Ergebnisse so zu erklären wäre, so kommt man glatt zur Ansicht, dass die Säurebildung nicht mit dem Eiweissabbau, sondern mit dem Eiweissaufbau gekoppelte Erscheinung sein soll.

Versuch 20.

Um die Ergebnisse des obenerwähnten Versuches an einem und demselben Blatt nachzuprüfen wurde die eine Hälfte des 6. Blattes von zirka 60cm. hohen Begonienpflanze Nr. 22. die 229 Tage lang im Gewächshaus künstlich gezogen wurde, am 5. IX. 1935 analysiert. Die eine andere üblich am Blattstiel belassene Hälfte wurde mit dem Stiel in dem mit 0.25% Asparaginlösung beschickten 250cc fassenden Kolben 2 Tage lang und dann weiter 1 Tag in destilliertem Wasser eingesteckt. Während des Versuches waren sie im zerstreuten Tageslicht bei *ca.* 18-22°C gestanden. Das Kontrollblatt wurde in gleicher Weise wie Versuchsblatt aber in destilliertem Wasser 3 Tage lang gebracht. Sie zeigten sich dazwischen keinerlei Schädigungserscheinung. Das Ergebnis an der Tabelle 23 zeigt, dass der Gehalt an Eiweiss-N trotz der Asparaginernährung abnahm. Der lösliche N. u. a. Ammon- und Amid-N nahm gegenüber zu, während der Amino-N fast unverändert blieb. Diese Ergebnisse könnten ganz ähnlich wie die beiden entsprechenden Versuchen mit Ammonsulfat- und Calziumnitratkulturen erklären werden. Und zwar der Eiweissabbau hat sicher mit der Verwundung an der Blattspreite zu tun, sodass die im Vergleich zum Kontrollblatt um 3 fach zuge-

Tabelle 23.

Der Säure- und N-Stoffwechsel in abgeschnittene Blattspreiten unter künstlicher N-Anreicherung

Bemerkung		Kontrolle		0.25% Asparagin		0.25% Harnstoff	
		Vor Versuch	Nach Versuch	Vor Versuch	Nach Versuch	Vor Versuch	Nach Versuch
mg. Oxalsäure in 1000g. Frischgew.		8319.3	+ 108.7	8929.5	+ 393.0	8939.1	+ 582.1
mg. N in 1000g. Frischgew.	Gesamt-N	2225.2	+ 49.3	3040.1	+ 124.5	2609.1	+ 1237.7
	Eiweiss-N	2071.4	− 8.2	2729.4	− 106.9	2361.6	+ 194.1
	Lösl.-N	153.8	+ 57.5	310.7	+ 230.4	247.5	+ 1043.6
	Ammon-N	55.4	− 0.6	86.3	+ 111.2	49.5	+ 108.1
	Amid-N	15.5	+ 11.9	73.9	+ 123.6	80.4	+ 451.2
	Amino-N	82.9	+ 46.2	150.4	− 4.4	117.6	+ 487.3
Amid/Ammon		0.28	0.50	0.86	1.00	1.62	3.37

nommene Säuremenge zum Teil von stark gesteigertem Eiweissabbauprodukte gebildet werden könnte.

d. Harnstofffütterung.

Im Pflanzenreich wurde das Harnstoff mit der Ausnahme bei den niedrigen Pflanzen bisher nie in grosser Menge angetroffen. Im allgemeinen häufen bei der Pilze die grössere Harnstoffmenge als bei den Samenpflanzen an: z. B. findet sie sich nach IWANOW in *Bovista nigrescens* 11.16%, in *Lycoperdon gemmatum* 10.70% und in *Psalliota pratensis* (Kulturrasse) 14.90% des Myzeltrockengewichts. Die erste Angabe über das Vorkommen von Harnstoff auch bei höheren Pflanzen stammt von WEYLAND, nach ihm das Carbamid (Harnstoff) als Stoffwechselprodukt der Wurzelpilze bei einer Reihe von mykotrophen Pflanzen nachgewiesen werden kann. Die WEYLANDschen Ergebnisse sind heute durch die Arbeit von WEISSFLOG sowie von KLEIN und TAUBÖCK widerlegt. Besonders häufig und in grossen Mengen (bis 0.7% der Trockensubstanz bei *Canavalia*) kommt Harnstoff in den Reihen der Rosales und Therebinthales. Kurz bevor die Arginase in Pflanzen von KIESEL entdeckt wurde, konnte TAKEUCHI die Anwesenheit des harnstoffspaltenden Enzyms Urease auch in höheren Pflanzen erweisen. KIESEL erweiterte die Zahl der Urease enthaltenden Pflanzen noch mehr, wobei die Bedeutung der Anwesenheit von Urease dortselbst aufgeklärt wurde. Die Uutersuchungen von TAKEUCHI und von KIESEL zeigten deutlich, dass die höheren Pflanzen oft ein viel grösseres Vermögen zur Harnstoffspaltung besitzen als die sogenannten Harnstoffvergärern unter den Bakterien, bei denen die höchste Harnstoffspaltung nur 46% des anwesenden Harnstoffs erreicht. Die von TAKEUCHI für Sojasamen gefundene Harnstoffspaltung erreichte 95%, die von KIESEL für Weizenkeime nachgewiesene ebenso 91%. Etwas später fand MARSCHALL für Sojasamen sogar 98%ige Harnstoffspaltung. Diese Befunde über die weite Verbreitung der Urease in höheren Pflanzen, insbesondere mit eiweissreichen Samen, wurden später durch eine ganze Reihe von Forschern bestätigt und erweitert. Die optimale Wasserstoffionenkonzentration der Ureasewirkung ist nach VAN SLYKE und seine Mitarbeitern sehr nahe am Neutralpunkt (pH = 7.0) liegt. Dieses Ergebnis wurde auch durch zahlreichen anderen Arbeitern, u. a. von LÖVGREN bestätigt. Der Optimalpunkt der Azidität ändert sich etwas mit der Harnstoff-Konzentration der Lösung, wie von VAN SLYKE gefunden und später von LÖVGREN an einem zhlreichen Materialien bestätigt wurde. Es ist also interessant zu untersuchen, wie die Harnstofffütterung den Säure- bzw. N-Stoffwechsel der sauren Pflanzen wie *Begonia* beeinflüssen kann. Sofern die Urease zur Wirkung kommt, muss sich eine Harnstoffernährung ebenso auswirken wie eine Ammonsalzernährung, sodass Harnstoff physiologisch zwischen einem Ammonsalz und dem Asparagin steht.

Versuch 21.

Zur Bestimmung des Säure- und N-Gehalts unter Harnstoffernährung kam die Blätter der 186 bzw. 201 Tage lang in Wasserkultur gezogenen Individuen Nr. 8 bzw. 16 und des einen aus Freiland genommenen zur Verwendung. Die Versuchsblätter waren 2 Tage lang mit dem Blattstiel in 0.25% Harnstofflösung und danach weiter 1 Tag in destilliertem Wasser gestanden. Die Analysenergebnisse durchschnittlich in Tabelle 22 zusammengestellt. Aus diesem Ergebnis ist ersichtlich, dass in Gesamtheit des ganzen Blattes alle N-Fraktionen an ihrer Menge stark zunahm, und dass sie in dieser Hinsicht die Ähnlichkeit zur vorstehender Asparaginkultur zeigt. Nur besteht zwischen beiden an ihrem Eiweiss-N-Gehalt ein Unterschied, indem die Eiweisssynthese bei Harnstoffkultur über doppelt so gross wie die bei Asparaginkultur stattgefunden ist. Dieses Unterschied in der Eiweisssynthese ist bloss dem Unterschied in der Tätigkeit der Blattspreite zuzuschreiben, weil der Blattstiel wie immer keine nennenswerte Rolle daran spielt. Andererseits in Bezug auf die Säurebildung hat die Harnstoffkultur, ganz ähnlich wie die Asparaginkultur, stark beschleunigenden Einfluss ausgeübt. Also diese Ergebnisse führen uns wenn die Säurebildung überhaupt mit dem Eiweissstoffwechsel zu tun hätte wie vorher zur Anschauung, dass sie nicht mit dem Eiweissabbau, sondern hauptsächlich mit dem Eiweissaufbau in innige Beziehung stehen soll.

Versuch 22.

Um wieder das oben erhielte Ergebnis an einem und demselben Blatt nachzuprüfen, wurde weiteres Versuch an einem Individuum Nr. 50 angeführt, was 220 Tage lang im Gewächshaus künstlich gezogen und schon ausgeblüht war. Die Blattspreite war noch grün und schien äusserlich ganz gesund. Die 4. Blattspreite wurde quer in zwei gleichen Teilen durchgeschnitten und die eine Hälfte sofort analysiert und die eine andere Hälbte stand mit dem Blattstiel 2 Tage lang in 0.25% Harnstofflösung und dann weiter 1 Tag in destilliertem Wasser. Das 5. Blatt desselben Individuums wurde als ein Kontroll mit dem Blattstiel, anstatt in 0.25% Harnstofflösung, in destilliertem Wasser eingetaucht. Der Versuch war den 5 IX. 1935 begonnen, den 8. IX. geendet. Die Analyse war bloss an Blattspreite ausgeführt. Die Analysenergebnisse sind in Tabelle 23 angegeben. Was dort augenfällig ist, würde erstens die enorme Vorsprung an Amid- und Amino-N, und zweitens die unerwartete Zunahme des Eiwiss-N sein. Weil im vorstehenden Versrch die Menge des Amid-N fast unverändert blieb, kann die hier gefundene Anhäufung des Amid-N nicht einfach zur Hemmungswirkung der sauren Eigenschaft des Versuchsblatteszurückgeführt werden. Vielmehr würde die verhältnismässig kleinere Zunahme (194mg.) des Eiweissaufbaues, im Gegnsatz zum Versuch mit dem ganzen Blattspreitse (658mg.) (Tabelle 22), einen grösseren Abbau neben dem zugleich bestehenden Aufbau beim Falle mit der Blatthälfte andeuten, sodass dadurch die enorme Anhäufung des Amino-N grössenteils zum Vorschein kam. Wenn die Sache so zu verstehen wäre, scheint die Amid-N-Anhäufung zum Teil mit der enormen Vorsprung der Amino-N-Menge, und zum Teil mit dem Einfluss der Blatthalbierung zu tun haben. Trotz dieser Eigentümlichkeit sind die Zunahme an Saure und Ammon-N ganz vergleichbar mit der bei der entsprechenden Asparaginkultur geblieben, sodass wir dort die Säurebildung vielmehr besser mit dem Eiweissabbau in Beziehung gestellt werden könnte.

e. Aminosäurefütterung.

Es ist heutzutage im allgemeinen bekannt, dass die totale Eiweisshydrolyse um etwa 80% des im Eiweiss vorhandenen N in Form der Aminosäuren wiedergibt, sodass die Aminosäuren den Baustein des Eiweissmoleküls bilden sollen. Unter den verschiedenen Aminosäuren der Pflanzeneiweisstoffe sind l-Asparaginsäure (α-Aminobernsteinsäure $COOH \cdot CH_2 \cdot CH(NH_2) \cdot COOH$) und d-Glutaminsäure ($\alpha$-Aminoglutarsäure $COOH \cdot CH_2 \cdot CH_2CH(NH_2) \cdot COOH$) vorherrschend.

Im Jahre 1926 haben QUASTEL und WOOLF nachgewiesen, dass die "ruhenden" Colibakterien l-Asparaginsäure enzymatisch im Ammoniak und Fumarsäure spalten: später WOOLF hat für das in den ruhenden Colibakterien vorkommende Enzym, welches Ammoniak aus der Asparaginsäure abspaltet, den Namen Aspartase vorgeschlagen. Unlängst VIRTANEN und TARNANEN isolierten aus *Bacillus* *fluorescens liquefaciens* Aspartase, welche sowohl aus l-Asparaginsäure unter Bildung von Fumarsäure Ammoniak abspaltet, als auch aus Fumarsäure und Ammoniak l-Asparaginsäure synthesiert. Sie stellen auch fest, dass die pH-Kurven der Spaltung und Synthese durch das Enzym gleich sind und das Optimum bei pH 7 bis 7.5 liegt; unter pH 5.5 kann das Enzym nicht mehr wirksam sein: die Aspartase ist viel säureempfindlicher als die Asparaginase. Die Spezifität der Aspartase ist ausserordentlich fein. Auf andere Aminosäuren — Glykokoll, Alanin, Leucin und Glutaminäure — wirkt das Enzym nicht. Auch die Derivate der Asparaginsäure scheinen nicht durch die Aspartase gespaltet zu werden. Nach ihren Untersuchungen ist die Aspartase im Pflanzenreich weit verbreitet und sich findet sowohl in den höheren Pflanzen — in Erbsenkeimlingen und jungen Gräsern — als auch in allen geprüften Bakterienarten, dagegen nicht in Hefe. Nun entsteht die Frage, wir die Säurebildung unserer Versuchspflanze unter N-Ernährung in Form der Asparaginsäure vor sich gehen wird. Sofern aber die Aspartase zur Wirkung kommt, muss die Kultur der sauren Pflanze mit Asparaginsäure die ähnlichen Einfüsse auf den Säure- und N-Stoffwechsel zum Vorschein kommen lassen wie bei der Kultur mit Ammonsalzen. Ob die Sache wirklich so ist kann erst durch den Versuch beantwortet werden.

Versuch 23.

Als Versuchsmaterial diente 3 Individuen, deren ein im Freiland und andere zwei in Wasserkultur herangezogen waren. Von je einer Pflanze wurden je 2 Blätter aufgenommen. Nr. 11 bzw. 16 war 193 bzw. 186 Tage lang in Wasserkultur gewesen und schon zur Blüte gekommen. Die Versuchsblätter standen mit den Stielen 2 Tage lang in der Lösung, die 0.25% Asparaginsäure oder Glutaminsäure enthielte; danach wurden sie weiter 1 Tag lang in destilliertem Wasser gebracht, um möglichst völliges Entfernen von Aminosäuren zu erreichen. Die Kontrollblätter standen dagegen mit den Stielen durchweg 3 Tage lang in den mit destilliertem Wassser beschickten 250cc. fassenden Kolben. Der Versuch wurde den 29. IX. 1935 begonnen, den 2. X. geendet. Nach dieser Behandlung waren die Blattspreiten ganz frischgrün wie vor dem Versuch und äusserlich keine Schädigung festzustellen. Die Analysenergebnisse sind in üblicher Weise in Tablle 24 zusammengestellt. Die Tabelle zeigt, dass hier der Eiweiss-N im Verhaltnis zu den obenerwähnten allen Fällen enorm zugenommen ist, und zwischen beiden Aminosäuren, in bezug auf die Zunahme des Eiweiss-N, gar kein Unterschied zu erkennen ist. Wir können also annehmen, dass hier die Erwartung bestätigt liegt vor in dem Sinne, dass die Aspartase durch die stark saure Reaktion unserer Blattzellen ihre Wirkung beeinträchtigt wurde. Damit parallel gehend zeigt die Tabelle noch weiter, dass sich die Eiweisssynthese nicht bloss in den Blattspreiten, sondern

Tabelle 24.

Der Säure- und N-Stoffwechsel in abgeschnittene Blattspreite unter künstlicher N-Anreicherung

Pflanzenteil		Blattspreite			Blattstiel			Blattspreite + Blattstiel	
Bemerkung		Kontrolle	0.25% Asparaginsäure	0.25% Glutaminsäure	Kontrolle	0.25% Asparaginsäure	0.25% Glutaminsäure	0.25% Asparaginsäure	0.25% Glutaminsäure
mg. Oxalsäure in 1000g. Frischgew.		5760.9	+ 501.2	+ 664.3	8562.8	+ 490.5	+ 570.7	+ 495.9	+ 617.5
mg. N in 1000g. Frischgew.	Gesamt-N	1700.5	+ 1081.6	+ 1103.3	415.9	+ 328.0	+ 463.4	+ 704.8	+ 783.3
	Eiweiss-N	1530.6	+ 766.3	+ 842.1	267.8	+ 111.5	+ 152.1	+ 438.9	+ 497.1
	Lösl. N	169.9	+ 351.3	+ 261.2	148.1	+ 216.5	+ 311.3	+ 265.9	+ 286.2
	Ammon N	40.4	+ 156.5	+ 120.1	41.7	+ 121.9	+ 179.5	+ 139.2	+ 149.8
	Amid-N	104.3	+ 101.4	+ 33.5	89.8	+ 45.5	+ 89.4	+ 73.4	+ 61.4
	Amino-N	25.2	+ 57.4	+ 107.6	16.6	+ 49.1	+ 42.4	+ 53.3	+ 75.0
Amid/Ammon		2.66	1.1	0.89	2.23	0.83	0.80		—

auch in den Blattstielen ansehnlich stattfand. (was wie in den vorstehenden Versuchen zu verstehen war). Alle diese Tatsache sprechen dafür, dass die beiden Aminosäuren als solche ohne beträchtliche Veränderungen in den Eiweissmolekülen eingebaut werden. Damit im Einklang steht auch die Tatsache, dass die Ammon-N-Zunahme ganz mit der bei den in vorigen Abschnitten behandelten Fällen vergleichbar geblieben ist. In Betreff der Amid-N-Zunahme steht aber dieser Fall zwischen den Ammoniumsulfat- bzw. Calziumnitrat- und den Asparagin- bzw. Harnstoffkulturen, was ebenfalls gegen den weitgehenden Abbau der zugefügten Aminosäuren spricht. Was nun die Säurebildung anbetrifft, so ist die zugenommene absolute Menge etwas weniger als bei den Ammoniumsulfat- bzw. Calziumnitratkulturen. Das scheint wahrscheinlich dahin zu deuten, dass wegen der Polymerisation der zugefügten Aminosäure zu den Eiweissmolekülen bei diesem Falle die Oxydation des Kohlenhydrates schwächer als bei den anderen sein konnte, die ja gewöhnlich zur Bildung organischer Säuren führt.

Versuch 24.

Um weiter die vorstehenden Ergebnisse an einem und demselben Blatt nachzuprüfen, wurden die folgenden Versuchen durchgeführt. Zum Versuch kam ein Individuum Nr. 43, das 250 Tage lang im Gewächshaus künstlich gezogen wurde und schon ausgeblüht war. Die Blätter waren schwarzgrün und äusserlich noch ganz gesund. Die Blattspreite wurde quer in zwei gleichen Teilen durchgeschnitten, deren eine Hälfte sofort analysiert wurde, während die eine andere Hälfte mit dem Blattstiel 2 Tage lang in der 0.25% Asparaginsäure- oder Glutaminsäurelösung.

und dann weiter 1 Tag lang in destilliertem Wasser stand. Die Kontrollpflanze Nr. 50 wurde 220 Tage lang ebenfalls im Gewächshaus künstlich gezogen deren Blätter ganz entsprechender Weise wie Versuchsblätter, anstatt in 0.25% Aminosäurelösung, in destilliertem Wasser standen. Die Versuche wurden den 5 IX. 1935 begonnen, den 8. IX. geendet. Die Analysenergebnisse sind in Tabelle 25 zusammengestellt. Beim Falle der Glutaminsäurekultur zeigt die Tabelle, dass die Sache in so fern ähnlich wie bei den vorigen erklärt werden kann, als die Eiweiss-N-Zunahme mit der Säurevermehrung begleitet wurde. Quantitativ gesehen ist aber die Erscheinung um so kompliziert, als die Eiweiss-N-Zunahme halb so gross wie bei den vorigen ausgefallen ist. Das heisst, dass hier das gleichzeitig vorgehende Gegenspiel des Prozesses, d. h. die Eiweisshydrolyse wegen der Verwundung kräftiger beteiligt hat. Dafür spricht auch die kräftige Amid-Anhäufung. Die doppelt so grosse Znnahme der Säure bei diesem wie bei den vorigen Fällen scheint damit in Einklang zu stehen. Was die Ergebnisse der Asparaginsäurekultur anbetrifft, so nahm hier Eiweiss-N im Gegensatz zur Glutaminsäurekultur enorm, und der Amino-N auch ziemlich ab, was zusammen dahin deutet, dass die Eiweisshydrolyse hier viel stärker als bei der Glutaminsäurekultur vorangegangen ist. Gerade dementsprechend wuchs die Säurezunahme so enorm, dass sie hier 3mal so gross wie bei der Glutaminsäurekultur emporgesteigen ist. Die Säurezunehme beträgt hier bei der Glutaminsäure- bzw. Asparaginsäurekultur 2 bzw. 6mal so gross wi beie den entsprechenden im vorigen Abschnitte. Diese Tatsache kann erst begreiflich sein, wenn man sich so

Tabelle 25.

Der Säure- und N-Stoffwechsel in abgeschnittene Blattspreite unter künstlicher N-Anreicherung

Bemerkung		Kontrolle		0.25% Glutaminsäure		0.25% Asparaginsäure	
		Vor Versuch	Nach Versuch	Vor Versuch	Nach Versuch	Vor Versuch	Nach Versuch
mg. Oxalsäure in 1000g. Frischgew.		8319.3	+ 108.7	8227.9	+ 1200.4	10331.7	+ 3103.4
mg. N in 1000g. Frischgew.	Gesamt N	2225.2	+ 49.3	2372.5	+ 427.7	2968.3	− 328.9
	Eiweiss N	2071.4	− 8.2	2156.3	+ 254.6	2557.4	− 343.9
	Lösl.-N	153.8	+ 57.5	216.3	+ 170.0	410.9	+ 15.0
	Ammon N	55.4	− 0.6	29.2	+ 84.9	145.0	+ 89.3
	Amid-N	15.5	+ 11.9	58.4	+ 130.3	82.2	+ 53.3
	Amino-N	82.9	+ 46.2	128.6	− 45.2	183.7	− 127.6
Amid/Ammon		0.28	0.50	2.00	1.65	0.57	0.58

verstellt, dass die wegen der Verwundung ausgelöste Eiweisshydrolyse neben der Eiweisssynthese, die meistens mit der damit innig gekoppelten Kohlenhydrathydrolyse zur Folge hat, direkt an die Säurebildung beteiligt.

B. Versuch an Blattspreiten und Blattstielen intakter Pflanzen.

Versuch 25.

Um die obenerwähnten Ergebnisse aus abgeschnittenen Blättern in den N-haltigen einfachen Lösungen zu kontrollieren, stellte ich dem folgenden Versuch an die intakten Pflanzen unter Verwendung der vollkommenen und zusammengesetzten Nährlösung an. Die Versuchspflanze Nr. 42 bzw. 48 war 202 bzw. 182 Tage lang in Kultur gewesen und schon ausgeblüht.

Als Versuchsnährlösung hielte ich mir folgende Stammflüssigkeit verrätig

	Kontroll-Nährlösung			N-reiche Nährlösung	
A	KH_2PO_4	28g.	A	KH_2PO_4	28g.
	KNO_3	28g.		KNO_3	56g.
	$MgSO_4 \cdot 7H_2O$	28g.		$MgSO_4 \cdot 7H_2O$	28g.
	H_2O	916cc.		H_2O	888cc.
B	$Ca(NO_3)_2$	112g.	B	$Ca(NO_3)_2$	224g.
	H_2O	888cc.		H_2O	776cc.

Zum Versuch wurde das Gemisch von 100cc. A und 100cc. B mit Wasser auf 2000cc. aufgefüllt und ein Spur von 10% $FeCl_3$ gefügt. In diesen Lösungen wurden die Pflanzen zum Versuch weiter 15 Tage lang kultiviert. Die Pflanze Nr. 42 diente als Kontroll- und Nr. 48 als Versuchsindividuum, wovon je 3 Blätter jedesmal zur Analyse abgenommen wurden. Nach der Frist der betreffenden Ernährung waren sowohl die Blätter des Kontrollversuchs als auch die des Ernährungsversuchs grün und schienen äusserlich ganz gesund. Die Analysenergebnisse der Säure- und N-Bestimmung sind in üblicher Weise in Tabelle 26 zusammengestellt. Diese Ergebnisse stehen im grossen und ganzen mit den Ergebnissen aus den unter Verwendung reiner einfacher anorganischer N-Verbindungen ausgeführten Versuchen mit abgeschnittenen Blättern in Übereinstimmung. Kleine Unterschiede sind aber zwischen beiden erkennen, indem bei den Versuchen an intakten Pflanzen die Eiweiss-N-Zunahme geringer und die Ammon-N-Zunahme viel grösser als bei den an abgeschnittenen Blättern zum Vorschein kamen, was vielmehr der Erwartung widersteht, weil man etwa die grössere Eiweiss- bzw. Ammon-N-Zunahme gerade bei den intakten Blättern zu erwarten wäre. Noch ein weiterer Unterschied ist daran zu erblicken, dass die Eiweisssynthese bei den Blattspreite intakter Pflanze nicht so enorm grösser als die bei den Blattstielen ausgefallen ist, wie es bei den Versuchen an abgesch-

Tabelle 26.

Der Säure- und N- Stoffwechsel in intakter Blätter unter 15tägiger künstlicher N-Anreicherung

Pflanzenteil		Blattspreite		Blattstiel		Blattspreite + Blattstiel
Bemerkung		Kontrolle	N-reiche Kultur	Kontrolle	N-reiche Kultur	N-reiche Kultur
mg. Oxalsäure in 1000g. Frischgew.		7913.6	+ 1453.0	9673.6	+ 1026.3	+ 1339.6
mg. N in 1000g. Frischgew.	Gesamt-N	2154.3	+ 296.1	689.3	+ 500.7	+ 398.4
	Eiweiss-N	1940.2	+ 69.6	394·3	+ 58.8	+ 64.2
	Lösl.-N	214.1	+ 226.5	295.0	+ 441.9	+ 334.2
	Ammon N	33.9	+ 218.7	71.4	+ 407.0	+ 312.8
	Amid-N	73.4	— 6.6	93.9	+ 52.9	+ 23.2
	Amino-N	106.8	+ 14.4	129.7	— 18.0	— 1.8
Amid/Ammon		2.79	0.38	1.22	0.31	-- —

nittenen Blättern der Fall war. Alle diese Tatsache sprechen dafür, dass bei den betreffenden Pflanzen dieses Versuchs grosser Wahrscheinlichkeit nach die Kohlenhydrate weniger als bei den vorigen Versuchen zur Verfügung gestellt wurden. Also bei vorliegendem Fall die inneren Bedingungen waren etwas anders als bei den vorigen Fällen, sodass die doppelt so grosse Zunahme der Säuremenge bei vorigen Fallen wie bei diesen etwa mit der kräftigeren, neben der Eiweisssynthese gleichzeitig vorgehenden Eiweissabbauprozesse teilweise in Zusammenhang gestanden sein könnte.

[Fortstzung folgt]

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ORIGIN OF POLARITY IN THE EGGS OF *SARGASSUM CONFUSUM* AG.[1]

BY

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(With 9 Text-figures)

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The purpose of this paper is to report when and how polarity is induced in ***Sargassum confusum***. It is well known that the axis of polarity in this alga is fixed early in the embryo stage, and that it is a product of still younger stages. So the question is when and how the polarity is bestowed in the first stage of development. There are many investigations on the mechanism for determination of the polar axis in the algae of the same family, i. e. by the direction of light rays (KNIEP, 1907; KNAPP, 1931; WHITAKER and LOWRANCE, 1936), by electric current (LUND, 1923), by the group effect (WHITAKER, 1937), by pH (WHITAKER and LOWRAENCE, 1937; WHITAKER, 1938), by heating (LOWRANCE, 1937), by the centrifugal force (KNAPP, 1931; WHITAKER, 1937), and by the entrance point of spermatozoid (KNAPP, 1931; ABE, 1941). The writer is of the opinion that although the axis of polarity may be determined by external factors as above mentioned, there still remains room to settle the problem of whether polarity is newly induced by these factors where there was no polarity or whether the change of the original axis of polarity existed before the influence of such factors. Herein the writer restricts his study to the origin of polarity.

The material was derived from ***Sargassum confusum***, a dioecious alga, which was growing on the northern shore of the chimney rock near the Marine Biological Station at Asamushi. Microscopic observations on the progress of maturation

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[1] Contributions from the Marnie Biological Station at Asamushi, Aomori Ken. No. 181.

of the male and the female were made daily before taking the necessary material. When it was considered to be two or three days before the egg and the sperm to be discharged the male and female were picked up one by one for experiment. The male plants collected were placed in a glass jar (20 cm. in diameter, 30cm. in height) without sea water and covered by inserting with a sheet of crumpled paper to retain moisture and prevent sultry, and the jar was placed in a dim corner of the laboratory. The female plants were cut into small branches (10. cm. length), thoroughly washed with filtrate dsea water, then placed in a small glass in pot (10 cm. in diameter, 20 cm. in height) filled with the same sea water and covered with a glass lid, these were set by a window facing the north.

On May 18th, egg and sperm were discharged. Some of the discharged receptacles were picked up from the female branch and put in a Petri dish with filtrated sea water and, on the other hand, a male receptacle was picked up and thrown into the female dish and left there for 10 minutes, thus artificial insemination was performed. Then the inseminated female receptacles were taken out and the eggs were scraped off with a sharp iron needle on a slide glass. In this waythe egg for experiment was prepared. All of the experiment were carried out at the room temperature, which was in the range of 18°±1° C, and in every case the sea water used was always after filtration through filter paper.

At the start of the experiment, the effect of some environmental factors, such as light, heat, gravity and pH were tested to determine their influence on the axis af polarity in the egg. To obtain satisfactory results the experiments should not be influenced by such factors as above mentioned. However, if the tests prove the aforesaid factors to have no effect on the axis of polarity in the egg, the experiment may be simplified. In this aspect, at first, the effect of light was tested.

Exp. 1. *The effect of light and heat.*

Fifteen plates of slide glass were prepared, on each of which were shown about 5,000 eggs directly after insemination. These were submerged in Petri dishes containing sea water, and then cultured in three lots: 1) in the dark room of complete darkness, 2) in the dark room illuminated sideways with 100W lamp from a distance of 30cm., 3) arranged under the diffused light in the laboratory.

On the following day, the cleavage took place in all of the lots, the axis of polarity was established, and a large cell group which was to become the main part of the embryo and a small lens-shaped cell destined to be divided into the first rhizoids were clearly differentiated. But, in every lot, the direction of the axis of polarity showed no difinite order but was arranged quite at random (Table 1). No difference could be detected in the mode and grade of the cleavage, among the three lots.

These facts seem to show: 1) the cleavage is not affected in its manner and speed by the existence or unexistence of visible light, 2) the direction of the axis of polarity is not affected by the direction of light rays or by the external gradient of temperature due to light. Owing to the above results, the following experiments were carried out without considering the state of light and temperature.

Exp. 2. The effect of gravity.

Eggs after insemination were taken with sea water into glass tubes and were rotated in an electric centrifuge for 60 minutes in the speed of 3,000 cycles per minute. After the centrifuging, they were immediately transferred to a Petri dish containing sea water.

A tricoloured stratification was produced in the egg by the centrifuging: a yellow cap of oil substance in the most centripetal region which was stained pink with Sudan III and black with osmic acid, then a brown zone of chromatophores and nuclei in the middle of the egg, and a vast colourless area of cytoplasm extending to the most centrifugal edge. No relation was found at this time between the direction of the stratification plane and that of the morphological axis of the egg, since the egg might be able to turn quite freely in the centifuging tube. Therefore it is not centain that there is any special light or heavy portion in connection with a definite morphological area of the egg. Since the zone of chromatophores stably fixed at the place and hardly returns to the original state of diffusion, and since the cleavage takes place in such a state of stratification, the direction of centrifuging may be easily found out in the later stage of development. Cleavage took place in the next morning, polarity was fixed, and the small primordial cell of rhizoids was developed. But the direction of the axis of polarity was quite different to that of centrifuging (Table 1).

The fact does not seem to show that what is essential in polarity is a specially light or heavy area which is fixed somewhere in the egg or is some partial accumulation of a light or heavy substance which is movable in cytoplasm. That is to say, it is not certain that the axis of polarity is affected by the gravitation. Accordingly, the gravitational environment may need not be taken into account in the following experiments.

Exp. 3. The effect of pH.

The egg after insemination were taken directly and cultivated in two ways: 1) divided into groups of twenty eggs for one group, and then sowed on slideglass, 2) put into glass capillaries of one egg for one capillary. The length of capillary was cut to 1 cm., and its inner diameter was made equal to the diameter of the egg including the outer layer of mucilage. The egg was settled in such a part of capillary as the distance to a mouth of the capillary was one third of the distance to the other mouth. Twenty such groups were prepared in the first lot, and twenty capillaries were provided for the second lot. All of them were submerged in Petri dishes containing sea water. Thus the effect of pH was tested.

Polarity was fixed and the primordial cell of rhizoids was formed in both of the lots. But, contrary to the expectation, the axis of polarity did not seem to be effected by the group or by the excessive difference of the distance to the mouth of capillary In these points, the present material is rather different from the egg of *Fucus* as was reported by WHITAKER (1937). As the eggs are breathing all the time and they are set in groups, their excreting CO_2 must be diffused or washed away in the outside of the group and be more concentrated in the inside; accordingly, the value of pH must be higher in the former and lower in the latter. Therefore if the axis of polarity is effected by pH, the rhizoidal pole should be developed in the same direction, centripetal or centrifugal within the same group. A similar interpretation may be allowed in the case of capillary. As the value of pH is higher in the shorter and lower in the longer distance from the mouth of the capillary, the rhizoidal pole must be formed always on the side nearer or further from the mouth of capillary if it be affected by pH. However such results were not obtained in either of the lots.

It was more clearly certified in a further experiment. Two glass capillaries, 50mm. in length, and 0.5mm. in the inner diameter, were prepared at first, one of them was filled with acidified sea water (pH 6.0) mixing HCl, and the other was filled with alkalified sea water (pH 8.8) mixing NaOH. Then a fertilized egg was placed between the two capillaries on a glass plate so that the mouth of each capillary was faintly in contact with the mucilaginous coat of the egg and the whole of the set was submerged into a dish of sea water. Twenty similar tests were prepared in the same way. Thus the egg was artificially exposed to H^+ on one side and OH^- on the opposite side, in other words, it was developed under the external gradient of pH.

Cleavage took place in 18 out of 20 attempts and the polarity became fixed with the formation of the primordial cell of rhizoids. Here also, the direction of the axis did not seem to be influenced by the external pH gradient (Table 1). Accordingly, the state of pH in the environment shall be disregarded in the following experiments.

Exp. 4. The original polarity and its succession.

The form of the egg immediately after being discharged is very variable, ranging from spherical to cuneate. 416 eggs were divided into four classes (Figs. 1–4), spherical, obovate, elongated and cuneate (Table 2)·

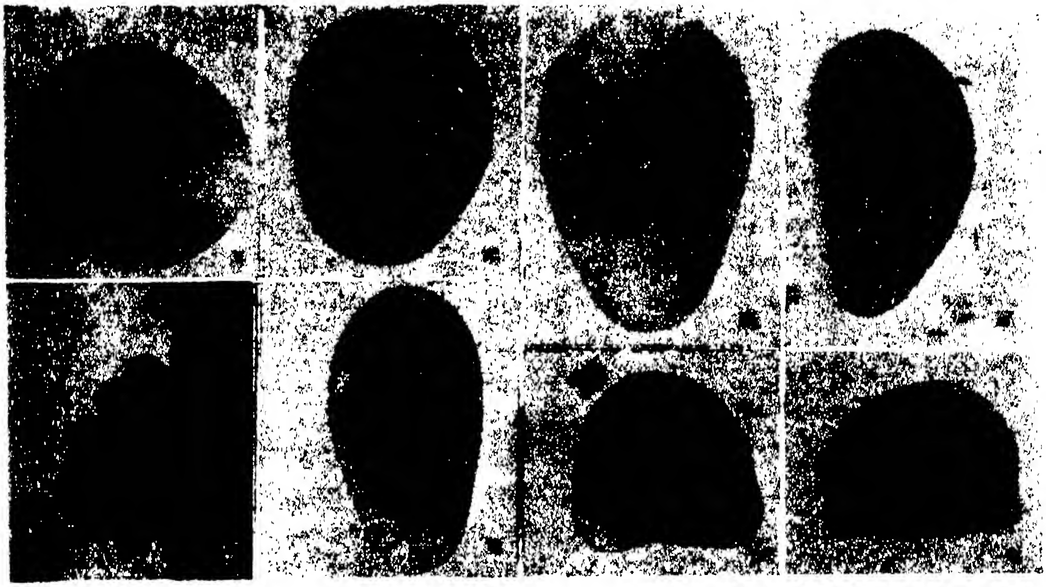
Table 1. Relations between the direction of the formation of rhizoidal pole and its number in various experiments.

Experimental conditions	Direction of the rhizoidal pole formation			
	North	West	South	East
Light	121 (light side)	136	107 (dark side)	118
Centrifuging	71 (centrifugal side)	87	110 (centripetal side	112
pH	4 (H+ side	5	3 (OH− side)	6
Succession of axis	100 (side of original peak)	0	0	0
Entrance point	100 (side of original peak)	0	0	0 .

Table 2. Variation of the form of eggs.

	Form				Total
	Spherical $\frac{\text{Length}}{\text{Width}}=1.0\sim1.2$	Obovate $\frac{\text{Length}}{\text{Width}}=1.3\sim1.5$	Elongated $\frac{\text{Length}}{\text{Width}}=1.6\sim1.8$	Cuneate $\frac{\text{Length}}{\text{Width}}=1.9\sim\infty$	
Number	58	316	78	9	416
Percentage	12.6	68.9	16.9	2.0	100

Observations of the different forms show that nearly 90 per cent of the total number of eggs are discharged with an ellipse form which is somewhat more pointed at the top of the major axis than at the opposite top of the same axis. That is to say, the majority of the eggs are already polarized in their form. Although it is rather difficult to obtain a perfectly spherical egg even in the group referred to spherical form, those belonging to this group seem to be more or less polarized in the same way. And even if an absolutely spherical egg is to be found it is quite probable that it is also polarized but with the axis of polarity extermely shortened. Similarly, the majority of the eggs are discharged with a morphological axis of polarity. Before being discharged, the eggs are not yet polarized and if some appear to be polarized, the axis is generally without definite direction, sometimes the peak points to the wall of the conceptacle, sometimes to the mouth, and sometimes to the lateral side. But, as soon as the eggs are discharged, they aquire a polarity and in a definite direction such as the peak points to the mouth of the conceptacle. The arrangement simulates a tassel (Fig. 5). Each egg is surrounded with a mucilaginous coat which is rather elastic and on the peaked side of the egg the coat is much elongated like a stalk, by which each egg is connected with the wall of the conceptacle through the small mouth. And as the stalks are bundled together by the small mouth of conceptacle, the eggs are quite intimately arranged like the strings of a tassel. In this way the axis of polarity is uniformly arranged in connection with the positional relation between the eggs and the conceptacle.



Figs. 1–4. Variation of the original form of the egg: 1) spherical, 2) obovate, 3) elongated, 4) cuneate. ×200. 5) Side view of the egg discharge. ×36. 6) Formation of the first membrane of cleavage. ×200. 7) A biaxial egg. ×200. 8) A biaxial embryo. ×200.

It seems that the origin of polarity at this time may be explained by mechanical relations among the softness of the egg, the elastic nature of the mucilaginous coat and the multiplicity of the egg discharge. That is, as the eggs are laterally pressed against each other in the egg, and as the degree of the lateral pressure is gradually increased towards the band of the tassel or the mouth of the conceptacle, it is very natural for the eggs to be narrowed and peaked in their portion nearer to the mouth of the conceptacle. This may be the origin of polarity in the egg of this alga. The polarity bestowed in this way is preserved as it is after the egg is separated from the conceptacle of the maternal body.

After fertilization, the egg is cleaved into two sister cells, one of them is round and plump but the other one is peaked (Fig. 5). The peaked cell next makes a small lens-shaped cell at the top, which is destined to be divided into the first rhizoids. The first membrane of cleavage at this time is accustomed to be formed at right angles to the original major axis (Fig. 6), which never changes but is succeeded through fertilization. In this way the primordial cell of rhizoids which was derived from the peaked cell is the successor of the peak in the original form of the egg. Namely, the rhizoidal pole takes its rise nowhere but in the peak which is placed nearer to the mouth of conceptacle at the time of discharge (Table 1).

The fact is more striking in the development of biaxial eggs. A number of biaxial eggs were derived (Fig. 7), each having two peaks branched from one head. Artificial fertilization was applied and the process of development was observed on these eggs. Two first membranes of cleavage for the two axes were formed simultaneously. And, in consequence, two primordial cells of rhizoids were formed one by one for the two peaks. Finally, a number of biaxial embryos having two rhizoidal poles were developed out of them, one of which is shown in Fig. 8. The process of this development is more useful to ascertain that the original axis is succeeded by that of the embryo.

Exp 5. The entrance point of spermatozoid.

It was reported in *Cystoseira barbata* by KNAPP (1936), *Coccophora Langsdorfii* and *Sargassum tortile* by ABE (1941) that the axis of polarity is determined by the entrance point of spermatozoid at the time of fertilization. However, it was doubted that such a law might hold for the present material which was a species allied to those algae especially to the last one. In other words, it is clear from the foregoing experiments that the axis of polarity is not changed but is succeeded through fertilization, thus it remains doubtful if there is any structural point in a definite portion in relation to the major axis, so that a spermatozoid can be taken in only through such a special point which may be the very cause of the unchangeability of the axis. If true, it may be nothing but a similar case of determination of polarity to those reported by the foregoing investigators so far as there is a similar relation between the axis of polarity and the entrance point, except for the only difference that the entrance point is prepared or unprepared.

For solving this doubt, an experiment was carried out according to the hint of MORGAN and TYLER (1938). At first, twenty glass capillaries were prepared with the inner diameter equal to the diameter of the egg including the mucilaginous coat. Then the egg was sucked up into the capillary, and the axis of the egg was variously adjusted in its direction so that it was kept in various angles to that of the capillary. Then the capillaries were pushed into a block of agar-agar and the insertion was stopped at the time when the egg was pushed back by the pressure of the agar block and returned to a portion 1 mm. from the opening mouth of the capillary. The capillaries arranged like this were kept horizontal and put into a Petri dish containing sea water together with the holding block of agar-agar. Insemination was done in such a condition.

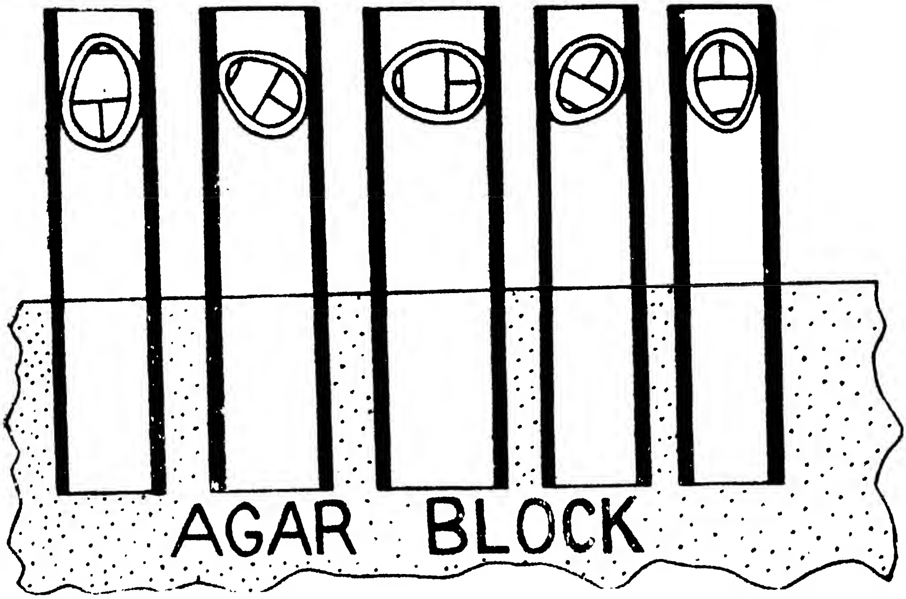


Fig. 9. Schematic illustration of the succession of the original axis independent of the entrance point of spermatozoid.

In consequence, all of them were equally fertilized and the cleavage took place in any of the eggs placed at various angles. However, the first membrane of the cleavage was formed not in connection with the axis of capillary but at random. And further, it was formed at right angles to the original axis of polarity just in the same way as in the natural state of cleavage. The primordial cell of rhizoids was cut out in the original peak. Thus the original axis of polarity was also succeeded in this experiment without being changed (Table 1).

Two inductions may be allowed here 1) It is probable that the spermatozoid can be taken in anywhere on the surface of the egg and a definite portion is not prepared in relation to the original axis for the entrance of the spermatozoid. Because fertilization takes place in all of the egg, while they are placed at various angles to the opened mouth of capillary and so the spermatozoid can be approached only at the side of the hemisphere of the eggs which is exposed to the mouth of the capillary and not at the side of the other mouth which is sealed with the agar block. 2) The axis of polarity does not seem to be determined by the entrance point of spermatozoid. Because the original axis is not changed by fertilization but is succeeded as in the original state of direction while the spermatozoid can intrude anywhere on the surface of the egg.

As is shown in the foregoing experiments the axis of polarity is rather stable in the egg of *Sargassum confusum* and does not change its direction by the environmental factors as in the case of *Fucus*, *Cystoseira*, *Coccophora*, and *Sargassum tortile*. Especially notable is that the arrangement of the inner materials has no power of determination against the axis of polarity. For the original axis is not changed while the inner materials can be easily disarranged by centrifuging and cleavage proceeds in such a state of disarrangement. Accordingly, it does not seem that the axis is determined either by the concentration of some light or heavy substances or by the partial distribution of some rhizoid making material, but that the axis of polarity is originally bestowed as a definite external form at the time of being discharged. The form is not a question of the internal parts but to the arrangement of the cortical substances, in which point, the determination of the axis of polarity in this plant seems to be somewhat agreeable with the theory of cortical cytoplasm presented by MOTOMURA (1935, 1935)

SAMMARY

1) The direction of the axis of polarity in the early development of *Sargassum confusum* is changed neither by the influence of the possible degree of light, heat, gravity and pH in the natural environment nor by the entrance point of spermatozoid, but it is originally determined at the time of egg discharge.

2) The original axis of polarity thus bestowed in the egg is not changed but is stably succeeded by the axis in the time of cleavage and further by the axis of embryo.

3) The essence of polarity in the first stage of development in this plant is not concerned with the arrangement of inner parts but with the original form of the cortical layers induced by the mode of egg discharge.

The writer express his cordial thanks to Prof. Dr. A. KIMURA for his supervision throughout the present studies.

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SCIENCE REPORTS OF THE TÔHOKU UNIVERSITY, 4TH SER.
(BIOLOGY), VOL. XVIII, NO. 3, 1950.

SOME ABNORMAL EMBRYOS OF *SARGASSUM CONFUSUM* AG. IN RELATION TO THE STUDY OF POLARITY.[1]

BY

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(With 12 Text-figures)

(Received August 31, 1949)

The writer obtained numerous abnormal embryos of *Sargassum confusum* in May of 1948 at Asamushi during observations on the development of eggs which had been inseminated artificially. Some of the most conspicuous embryos found are: 1) one-cell embryos (Figs. 5 to 8) having one or more rhizoids which were raised directly after fertilization without cleavage; 2) two-cell embryos (Fig. 9) having one large or small branch rhizoids ramifying from a common rhizoidal cell raised by the growth of the two sister cells after the first cell division of the egg without further progress of cleavage; 3) sparse-cell embryos (Figs. 10) in which rhizoids were raised before the egg reached to the normal stage of raising rhizoids (Fig. 3) and the cleavage ceased in such a stage; 4) over-cell embryos (Figs. 11 and 12) with rhizoids raised by the growth of some composing cells at the peak after the egg was cleaved into so many descendant cells in which the usual stage of raising was not passed.

In any example of the abnormal mode of raising in rhizoids in these embryos, a notable nature is that they are raised always in the peak of an egg which has been pointed since it was discharged from the conceptacle. The facts suggest the following four interpretations.

1. The direction of polar axis is latent before cleavage.

In the normal course of development, an egg is first divided into two sister

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[1] Contributions from the Marine Biological Station at Asamushi, Aomori Ken. No. 182.

cells (Fig 2). then at the edge of one sister cell, which is somewhat more peaked than that of the other, a small lens-shaped cell is cut out, this is destined to be the primordial cell of rhizoids (Figs. 3 and 4). In those eggs, however, whose cleavage was hindered in any cause (Figs. 5 to 8), the peak is lengthened continuously till it becomes one or more rhizoids; the projection is always limited to the peak. This phenomenon is a general feature not only in these one cell embryos but also in the other cases. From this fact, it is believed that the axis of polarity is not bestowed after cleavage but is latent early in the egg. Latent polarity only appears in the later stage of development in the normal course. That is to say, the axis is not due to the internal structures of an egg, or to the state of arrangement of descendant cells by cleavage, but is rather latent in the external form as a whole. whose side is more peaked.

2. The appearing form of polarity is latent before cleavage.

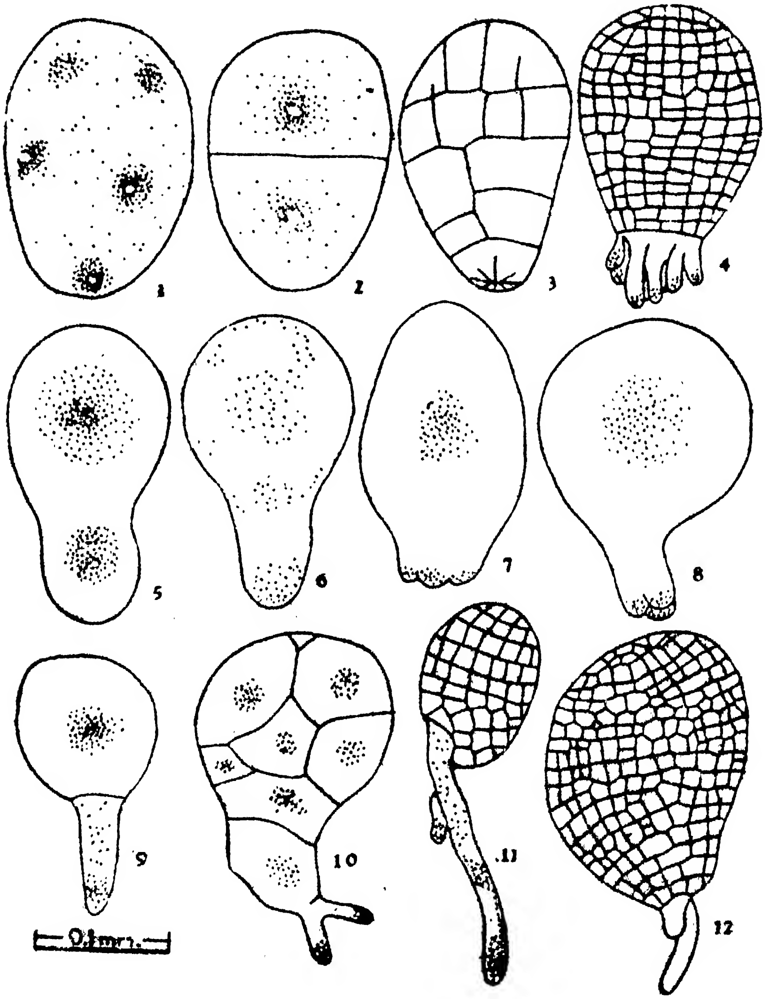
In the normal course of development, the primordial cell of rhizoids formed in the peak is usually radially divided into eight sister cells (Fig. 3), which later become rhizoids (Fig. 4). But in a noncleaved abnormal embryo composed of only one cell (Figs. 7 and 8), the rhizoid is able to be ramified into two or three branch rhizoids, thus appearing as formed in anormal embryo. Similar examples may also be seen in two or sparse cell embryos. That is, it seems that the appearing form of polarity or the ramifying nature of rhizoid is bestowed neither by the total amount of the individual functions of the descendant cells in a body derived through cleavage nor by a special order of arrangement of such cells. but is latent in the egg as a whole, quite freely from the manner of cleavage but also from an egg's condition cleaved or uncleaved.

3. A hindrance to cleavage does not always hinder the act of polarity.

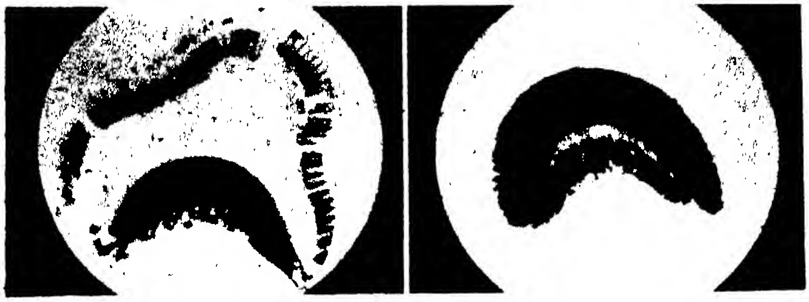
The time of raising rhizoids in the normal course of development is in the stage of about ten to fifteen cells (Fig. 3). However, many exceptional cases are found in the abnormal embryos. That is. even though the normal progress of development may be hindered in one or another stage, rhizoids can be raised in such a stage of hindrance. Hindrance to cleavage does not hinder rhizoid raising nor the act of polarity. They can be raised in a voluntary stage, 1) before cleavage reaches the normal stage of raising. or 2) after exceeding the normal raising time.

As above mentioned, rhizoids may be raised in the same direction and form of appearance in any stage of development. That is, each stage of development proceeds with a constant drift, a drift for the appearance of the latent polarity, in a definite form, and in a definite direction.

The writer's sincere thanks are due to Prof Dr. A. KIMURA for his kind advice in the course of study.



Figs. 1–4. Normal course of develpment. 5–8. one-cell embryos. 9. Two-cell embryo. [10. Sparse-cell embryo.] 11.12. Over-cell embryos raising rhizoids in the later stage which exceeds the normal time of raising.



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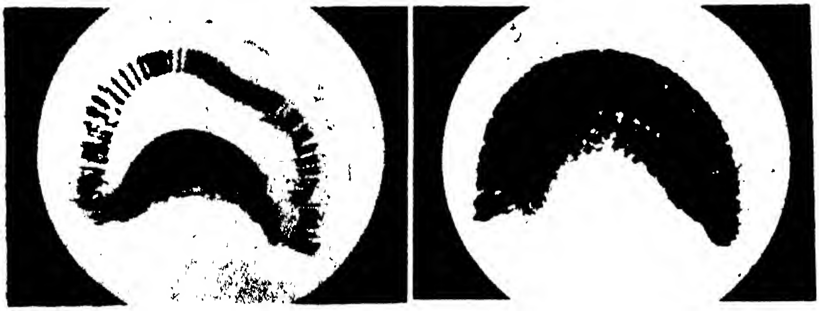

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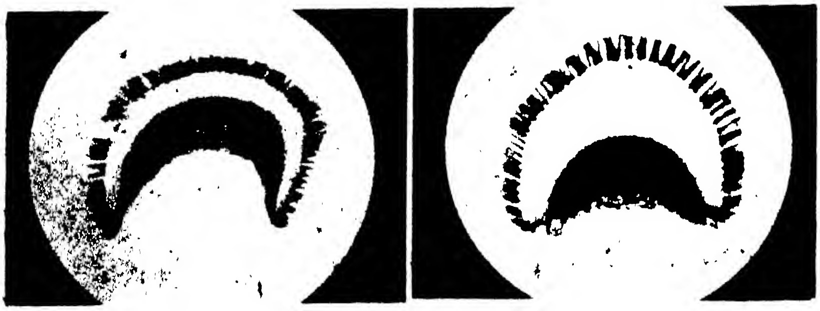
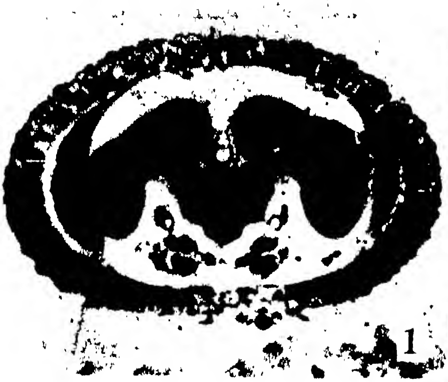


DD (8°C) ND (8°C)

The positions of three retinal pigments in the various conditions.

DD. Day dark eye. DL. Day light eye
ND. Night dark eye. NL. Night light eye
DD (8°C.) Day dark eye exposed at 8°C.
ND (8°C.) Night dark eye exposed at 8°C.

S. SATÔ: COMPOUND EYES IN MOSQUITOES. I. PL. V.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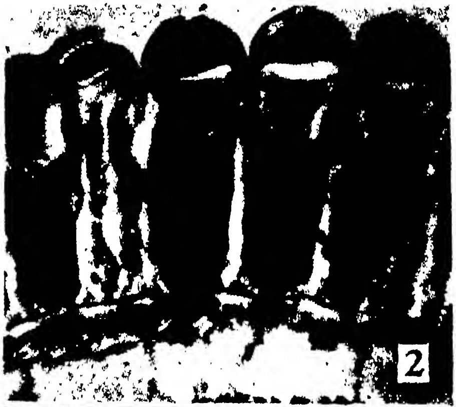
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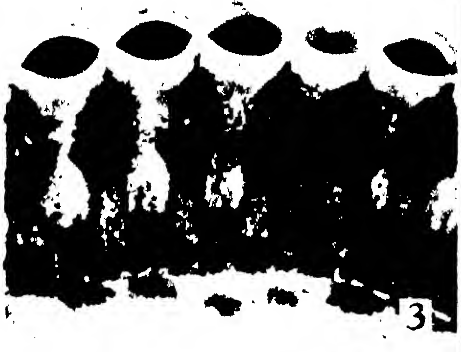
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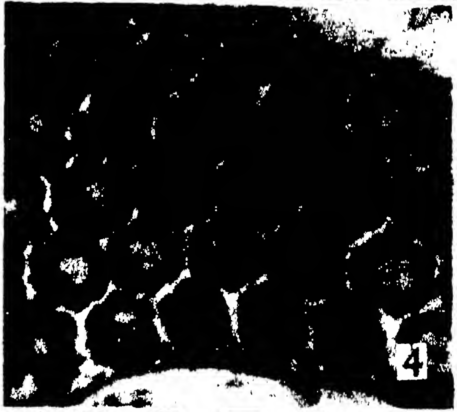
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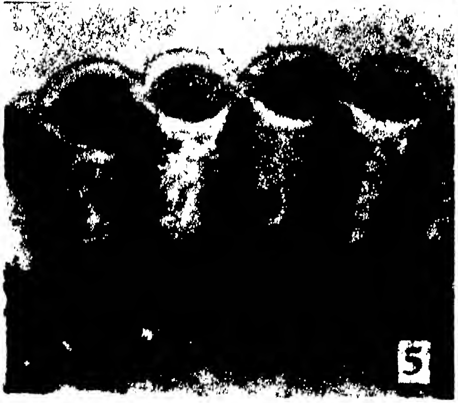
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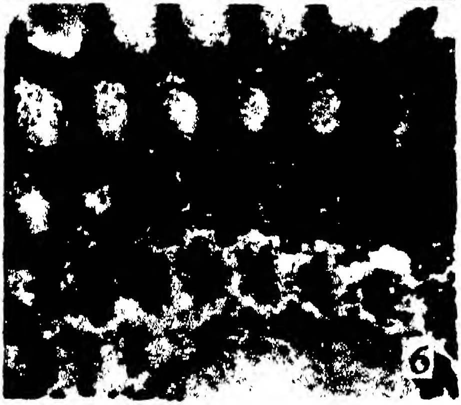
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ERRATA

Vol. XVIII

P. 318, line 20 from top, for miuutes read minutes

P. 320, in Table 2, for 98.7 read 68.7

P. 321, line 15 from top, for liqid read lipid

P. 333, in Table 2, for 2105 0 read 210570; for 925065 read 295065

P. 334, in Table 3, for 6 5~675 read 615~675

P. 351, line 5 from bottom, and p. 359, line 6 from bottom, for including actinomycetes read including few actinomycetes

P. 353, line 12 from top, for inclusive of actinomycetes read inclusive of few actinomycetes

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STUDIES ON THE PHYSIOLOGY OF CILIARY MOVEMENT
IV. EFFECT OF ACETYLCHOLINE AND ESERINE*)

By

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(With 2 Text-figures)

Since the work of A. LOEWI 1921, the physiology of nerve and muscle encountered a profound revolution by the introduction of concept of chemical transmission of the nervous excitation. The works of the schools of LOEWI, DALE and CANNON have elucidated that Vertebrates possess two kinds of nerves characterised by the nature of the chemical substances liberated at their terminations: the cholinergic nerves liberate acetylcholine and the adrenergic nerves liberate adrenaline. Acetylcholine and adrenaline have thus gained great importance in recent physiology (BACQ 1937).

In the previous paper the senior author of the present paper reported the results of the experiments on the effect of adrenaline upon the ciliary movement of the oyster gill (NOMURA 1937). It was found that adrenaline inhibits the ciliary movement of the excised gill tissue of the oyster, *Ostrea gigas* THUNBERG, at any concentration thus far examined.

Acetylcholine has been found in various molluscs and invertebrates and its action on various tissues and organs of different invertebrates has been studies by many workers. Choline esterase, which hydrolyses acetylcholine, has also been found in various molluscs and other invertebrates and its action has been studied. Adrenaline and acetylcholine are antagonists, and eserine, or physostigmine, inhibits choline esterase. These agents have been studies generally in connection with nervous and muscular activities. It would be interesting to study their actions upon activities of other kinds of living systems, *i.e.* ciliary movement, and cell activities in general. It may here be pointed out that there are two kinds of ciliary movement; one is under nervous control as in the ciliated epithelium of the palate of the frog (SEO 1931) and in the velar cilia of Nudibranch veliger, which cause locomotion of the larvae (CARTER 1926), the other kind of ciliary movement is not controlled by the nervous system and continues almost

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*) Contribution from the Oceanochemical Institute of the Tôhoku University, Onagawa, Miyagi Ken, Japan.

incessantly as far as the conditions are favorable, as in the gill of bivalves. The ciliary activity of the epithelial cells of the gill of the oyster, *Ostrea gigas*, is very persistent and continues movement almost as far as the ciliated cells are alive, and can, therefore, be taken as the criterion of survival of the cells after exposure to toxic substances (NOMURA and IMAI 1936). The ciliated cells of the gill of bivalves are devoid of innervation as far as we are aware (LUCAS 1931 a, b). GRAY 1928 compares the cardiac muscle and cilia and points out their similarity, but our experimental results show discrepancies as far as actions of acetylcholine and adrenaline are concerned.

The statements above made all indicate the significance of the present work and further discussion will be made subsequently under a separate heading.

MATERIAL AND METHOD

The material and method of the present work is the same in essential as that of the previous work on adrenaline (NOMURA 1937). Pieces of proper form and size were made from the gill tissue of the oyster, *Ostrea gigas* THUNBERG. The gill pieces thus prepared were allowed to crawl in a glass tube, provisionally called a "measuring tube" (NOMURA 1937, fig. 1). The gill piece proceeds in the tube, with the ventral margin behind, at a quite uniform velocity as long as the conditions remain unchanged. The velocity was measured every minute, as a rule, by reading the scale on the bottom of the tube graduated in millimetres. Each arm of the tube has graduations of 500 millimetres. The velocity of each gill piece in normal sea-water was taken as the standard, and that in experimental solution was expressed in percentage of the normal velocity. The slightest disturbance may interfere with the velocity and care should be taken to avoid it as far as possible. The measuring tube consists of two arms jointed with each other at a right angle, provided with a well ground glass cock. The bore of the cock is equal to that of the arms, and allows the gill piece to glide smoothly in and out of the cock; otherwise the unevenness of the bottom surface of the arm and cock would hinder smooth progress of the gill piece. The first arm of the measuring tube contains the normal sea-water and the second arm contains the experimental solution: various desirable concentrations of the drug in sea-water. The availability of the velocity of the gill piece crawling in the measuring tube as the measure of the ciliary activity was carefully worked out in our previous papers (NOMURA 1937; NOMURA and TOMITA 1933; TOMITA 1934 a, b), and for the details of the method the reader is referred to those works. The cock is open to the first arm at first, and when the piece has entered the bore of the cock, it is gently turned a right angle and is opened to the second arm of the measuring tube so that the gill piece can crawl out smoothly into the second arm, without being disturbed from without. The gill piece should be handled very gently and should not be picked up with forceps. When the gill piece is to be transferred from sea-water or a solution to another, it is allowed to glide on a small perforated spoon, specially prepared for this purpose, and then allowed to glide into the new solution.

Acetylcholine chloride, SCHERING-KAHLBAUM power preparation, in ampulla, was dissolved in sea-water in concentration of 1 : 100 by weight and kept as stock solution until use, and later diluted to desired concentrations when needed. Acetylcholine is decomposed by alkalies, and sea-water is weakly alkaline (pH 8.2), so the freshness was preferrable and the stock solution was prepared anew several times, but the experiment extended over a long period and the strength of the solutions may not have been uniform and caused irregularities in the results of experiment. This may be also the case with eserine. As to the irregularities of the results of experiments, the seasonal variations of the ciliary movement of the gill of the oyster may be mentioned. It was quite recently discovered that ciliary activity of the oyster gill greatly decreases after spawning in summer, and the decrease continues for a long period, and recovery begins in winter (USUKI and NAKAJIMA, *p. c.*).

EXPERIMENT

1) *Acetylcholine.*

Experiments were carried out in different seasons over three years and this might be also the cause of diversity of the results of experiment. The temperature varied also in a wide range during the whole period of experiment, but it fluctuated within the limit of ± 0.5°C. in each experiment. The results are summarised in the tables and figures, and some comments will be given in addition.

The highest concentration of acetylcholine examined was 10^{-3} which was proved to be decidedly inhibitory. The velocity of gill piece gradually decreased and became nul, but in some case it remained over 50% for more than 15 minutes. One piece, after complete stoppage in solution of concentration 10^{-3}, recovered 71.5% of its initial velocity in less than 20 minutes, when returned to normal sea-water. The penetration of the agent seems to be quick and the effect, inhibitory or accelerative, could be determined long before the gill piece reached the end of the second arm of the measuring tube. At the concentration of 10^{-6}, it was observed that after depression of ciliary movement down to 82.5% of the

Table I. Effect of Acetylcholine

		Concentration	10^{-3}	2×10^{-4}	10^{-4}	10^{-5}	2×10^{-6}	10^{-6}	2×10^{-7}	10^{-7}	10^{-8}
		No. of exp.	3	3	11	7	9	9	5	7	1
Velocity	Normal	Maximum, mm/sec.	56.3	39.4	49.4	47.8	33.8	47.2	36.2	38.5	24.1
		Minimum, mm/sec.	33.1	21.4	25.8	28.6	18.6	24.7	17.9	21.9	24.1
		Average, mm/sec.	45.2	32.9	36.3	34.5	27.13	31.5	25.8	32.6	24.1
	Experimental	Maximum, %	49.4	49.2	129.0	105.5	155.0	120.0	101.1	139.0	105.3
		Minimum, %	0	0	0	82.5	39.6	93.5	91.2	97.0	105.3
		Average, %	16.4	31.1	79.8	94.3	100.7	105.3	96.8	117.3	105.3
		Temperature °C.	23~24.5	22.6~23	18~24.5	19.5~27	18~21.0	[illegible]~26.5	18~22.1	18~26	18.[illegible]

normal, the supernormal recovery of 115% was made, when the gill piece was returned to the normal sea-water. At the concentration of 2×10^{-7}, the variation was not great and the average velocity was 96.8%, *i.e.* slight inhibition. It is, however, doubtful whether this is the true effect, as the lower and higher concentrations, 10^{-6} and 10^{-7}, both are accelerative, and the value of the velocity at 2×10^{-7} alone deviated far from the curve (Fig. 1). The most of the experiments with these concentrations were carried out in the same season. The effect of concentration 10^{-7} was quite accelerative, and the average velocity at this concentration was 117.3%. The effect of concentration of 10^{-8} could be examined only in one case and was proved to be slightly accelerative. The more di-

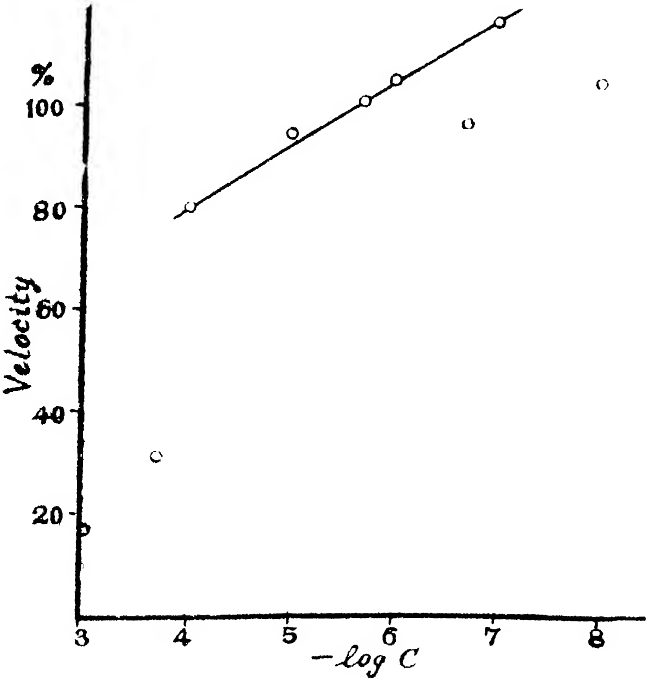

Fig. 1. Activity-concentration curve of effect of acetylcholine on cilia. Ordinates: Velocity of ciliary crawl. Abscissae: −*log* concentration of acetylcholine.

lute concentrations might not be expected to give definite results beyond the limit of experimental errors, and were abandoned to be examined.

From the results of experiments thus far performed, it is clear that the effect of acetylcholine is inhibitory in higher concentrations and accelerative in concentrations from 10^{-6} to 10^{-8}. For the range of concentrations of 10^{-4} to 10^{-7}, the relation of ciliary activity plotted against *−log* concentration of acetylcholine is linear, and it is worth noting that both acceleration and inhibition are represented upon a straight line. Whether the apparently straight line makes a part of a continuous smooth curve or not is an open question.

2) *Eserine.*

Eserine, or physostigmine, an alkaloid, inhibits the action of choline esterase which hydrolyses acetylcholine and obliterates its prolonged action. This action of eserine is indispensable in the neuromuscular mechanism, because the accumulation of acetylcholine would cause an excessive activity of muscles. Both acetylcholine and eserine are found also in molluscs and other invertebrates and their action in these animals have been studied. The effect of eserine in ciliated cells would be interesting from a viewpoint of general physiology.

Eserine (Merk's preparation) was dissolved in normal sea-water at concentrations of 10^{-3} to 10^{-8}. At concentration 10^{-3}, the ciliary movement was unexpetedly high, probably due to experimental error. The effect of other concentrations could be plotted against the *− log* concentration as a smooth curve (Fig. 2). At the concentrations 10^{-6} and 10^{-7} the effect is distinctly accelerative, while at the concentration of 10^{-5}, the velocity of the gill piece might temporarily exceed the normal or fall to zero. The maximum of the curve lies near 10^{-7}.

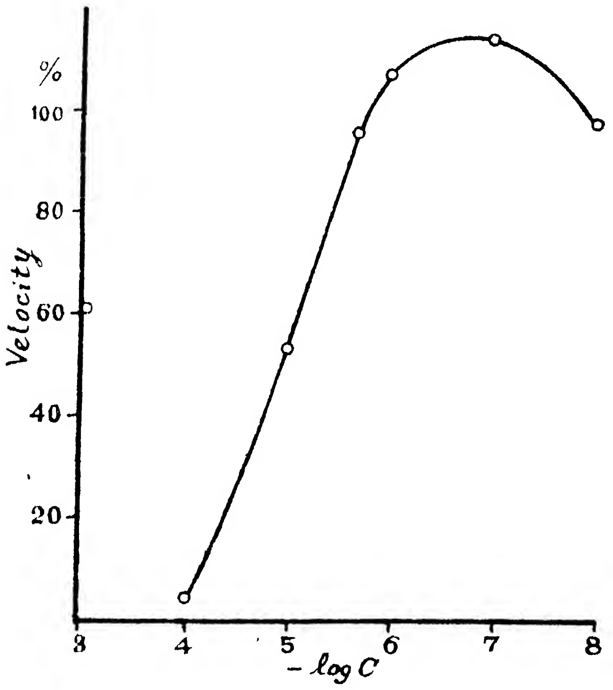

Fig. 2. Activity-concentration curve of effect of eserine on cilia. Ordinates and abscissae similar to those in Fig. 1.

Table II. Effect of eserine

		Concentration	10^{-3}	10^{-4}	10^{-5}	2×10^{-6}	10^{-6}	10^{-7}	10^{-8}
		No. of exp.	2	6	4	1	3	3	2
Velocity	Normal	Maximum, mm/sec.	39.4	35.9	43.1	44.2	33.9	48.5	47.5
		Minimum, mm/sec.	29.6	18.9	32.7	44.2	19.5	18.9	42.1
		Average, mm/sec.	34.5	28.2	36.1	44.2	24.7	35.7	44.8
	Experimental	Maximum, %	81.6	24.1	106.7*	96.6	126.7	132.8	99.1
		Minimum, %	41.6	0.0	0	〃	93.2	102.5	95.9
		Average, %	61.6	4.0	53.5	〃	107.3	114.3	97.5
		Temperature °C	19.6~19.7	19.0~21.6	20.7~21.5	20	19.5~19.6	21.3~22	22.5

* Temporary velocity 139%.

3) *Acetylcholine and eserine combined.*

In vertebrate experiment, eserine is usually used to inhibit the decomposition of acetylcholine by choline esterase in order to make evident the effect of acetylcholine. In the present work also, eserine was used in various combinations with acetylcholine. They were used in most promissing concentrations, but eserine did not appreciably improve the effect of acetylcholine. In three cases out of four, slight acceleration was observed, while the remaining case showed 92.2% activity, *i. e.* slight inhibition.

Table III Effect of Acetylcholine and eserine

No. of exp.		Concentration a. c.*	Concentration Eserine	Normal velocity mm/sec.	Exp. velocity mm/sec.	Exp. velocity %	Temperature
II	9	$5/6\times10^{-6}$	$1/3\times10^{-6}$	31.8	32.4	101.8	21.5°
〃	3	1.8×10^{-6}	2×10^{-6}	42.6	44.7	104.9	20.0°
〃	6	2×10^{-7}	2×10^{-7}	38.7	41.2	106.5	18.5°
〃	4	1.8×10^{-7}	2×10^{-7}	37.6	34.7	92.2	20.0°

* a. c. = acetylcholine.

DISCUSSION

Acetylcholine and choline esterase have been found in many tissues and hemolymph of various molluscs and other invertebrates (Bacq 1937, 1947), but the molluscan tissues are not always very sensitive to acetylcholine. The minimal active concentration of acetylcholine is 1×10^{-6} for the ventricle of *Ostrea edulis* (JULLIEN 1936). The action of eserine may be absent or present in different molluscs (BACQ 1947), and all the attempts for demonstration of cholinergic nerves in molluscs, except in *Venus mercenaria*, have failed.

Our experiments show that acetylcholine and eserine inhibit the ciliary movement of the excised gill of the oyster in higher concentrations, and accelerate in lower concentrations (10^{-6} to 10^{-7}), but the acceleration is not so remarkable. Simultaneous action of acetylcholine and eserine does not apparently promote the action of acetylcholine. Various substances, having no specific action, may excite or inhibit different activities according to the concentration of their solutions. The results of our experiments here presented are in accord with the well known general fact in pharmacology which is formulated in the ARNDT-SCHULZ Law: "Weak stimuli excite, medium stimuli partially inhibit and strong stimuli produce complete inhibition." (CLARK 1933, p. 195). From these facts, the present authors rather would maintain that acetylcholine and eserine and their combination might not have specific action on the ciliary activity of the oyster gill.

LUCAS studied the innervation of the gill of *Mytilus edulis* and other bivalves and reports absence of innervation of ciliated cells of the gill (LUCAS 1931 b). ISHIKAWA 1927, ISHIKAWA and OHZONO 1931 studied the effect of various drugs upon the ciliary movement of the palatine and pharyngeal epithelium in vivo and in vitro. Adrenaline was effective or ineffective, and when effective, it was only temporarily excitatory. This is in accord with the results of our previous work on adrenaline. The ciliated cells in the palatine mucous membrane of the frog are innervated by accelerator nerve fibres contained in Nervus facialis, N. trigeminus and N. glossopharyngeus, and the ciliary movement is accelerated, under normal conditions, reflexly. (SEO 1931). Some authors declare the presence of inhibitory nerves of ciliated cells, but SEO denies it. (SEO 1937). The fact observed by ISHIKAWA and OHZONO, that adrenaline is ineffective or only temporarily ffective, and acetylcholine is definitely accelerative, is in accord with the assertion by SEO. It seems highly probable that acetylcholine and adrenaline, sympathicomymetic and parasympathicomymetic drugs, require, after all, for their action, the presence of sympathetic and parasympathetic nerve fibres.

In the ciliated cells of the gill of the oyster, adrenaline was not accelerative on the average, and acetylcholine was slightly accelerative, but acceleration was not pronounced and might show nothing more than the fact expressed in the ARNDT-SCHULZ Law (*vide supra*). In the oyster gill also adrenaline and acetylcholine would require the presence of adrenergic and cholinergic nerves for their action, as the ciliated cells of the gill are devoid of innervation of any kind.

Accelerative effect of acetylcholine and eserine are not pronounced and seems to be fortuitous and not specific for the ciliary activity.

CLARK deals with various problems of the mode of drug action in his excellent book and gives innumerable formulae and curves describing the mode of drug action, but none of these seems to fit the cases here reported. For the kinetics of the action of acetylcholine and eserine, experimental data should be much more enriched, and we would not venture theoretical treatment of the subject on this occasion.

TAKATSUKI studied the effect of adrenaline and acetylcholine upon the heart of the oyster. The former agent increases tonus and frequency of the heart, and the latter at the concentration of 10^{-3}, arrests the heart in systolic condition (TAKATSUKI 1933, 1949; TAKATSUKI and MATSUI 1939). GRAY compared the heart and cilia and pointed out their similarity. But the facts observed by TAKATSUKI on the heart of the oyster and by the present authors on the cilia of the gill of the allied species are quite contradictoroy to GRAY's assertion, at least as far as actions of these drugs are concerned. As to the effect of adrenaline on the ciliary movement of the gill of the oyster, *Ostrea gigas*, the more detailed discussion was given in the previous paper (NOMURA 1937). SHMAGUENA 1935 reported inhibitory effect of adrenaline and sympathetic nerves and accelerative effect of the vagus. SCHMAGINA 1936 also observed inhibitory effect of the sympathetic and adrenaline, and accelerator effect of the parasympathetic on the ciliated epithelium of the oesophagus of the frog and on the trachea of the cat and the dog. Adrenaline lowered ciliary activity and this effect was diminished by atropine. Besides automacy of the ciliary movement, therefore, there is also a nervous acceleration and inhibition. But the relation of the sympathetic and parasympathetic nerves and acetylcholine and adrenaline to ciliary movement is just the reverse of their relation to the heart activity.

SUMMARY

1). The effect of acetylcholine and eserine on the ciliary movement of the gill of the oyster, *Ostrea gigas*, was examined.

2). Acetylcholine and eserine inhibit ciliary movement at higher concentrations, and acetylcholine accelerates at concentrations of 10^{-6} to 10^{-8}, and eserine accelerates at concentrations of 10^{-6} to 10^{-7}.

3). The fact just mentioned, together with previous observation (NOMURA 1937), show that the effect of acetylcholine and adrenaline on ciliary movement in the oyster is just the reverse of their effect upon the heart in general.

4) The above statement is in accord, from a viewpoint of neurohumoralism, with the fact that sympathetic inhibits and the parasympathetic accelerates the ciliary movement of oesophagus and trachea in vertebrates.

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PHYSIOLOGICAL STUDIES ON THE PIGMENTARY SYSTEM OF CRUSTACEA. VI. THE OXYGEN CONSUMPTION OF A SHRIMP *PARATYA COMPRESSA* UNDER EXPERIMENTAL CONDITIONS[*][**]

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(With 3 Text-figures)

INTRODUCTION

NÄGELI (1893) estimated the amount of copper present in 12 litres of distilled water, which had been four days in contact with 12 two-pfennig pieces. It contained one part in seventy-seven millions. This water was powerfully toxic to *Spirogyra*, killing it in one minute. On account of the very small quantity of copper in the water, NÄGELI gave the name of "oligodynamic" to the action in question. Since then, many workers have found that solutions of copper, silver, mercury and gold are toxic to bacteria and fresh water animals and plants at dilutions. No satisfactory explanation has been advanced for the high toxity of these metals at great dilutions, and there has been some discussion as to whether these metals exert their poisonous effect in the colloidal or the ionic state.

COOK (1926) stated that copper chloride solutions caused a rapid decline in the respiration rate of *Aspergillus* and *Nitella*. Since, it may be suggested that the oligodynamic action of certain heavy metals is the result of the destruction or inactivation of substances essential to cellular respiration. JONES (1941) has reported on the effect of ionic copper on the oxygen consumption of *Gammarus pulex* and *Polycelis nigra*.

The present author has attempted the investigation of the oxygen consumption of a shrimp under the effects of heavy metals, comparing with the respiration rate under normal conditions. The work has been carried out at the Biological Institute in Sendai, in 1943.

I am grateful to Prof. S. NOMURA for his kindness shown during the course of the present study.

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[*] This report is a part of the series of papers submitted to the Faculty of Science, Tôhoku University, in partial fulfilment of requirements of the degree of Doctor of Science, March 1948.

[**] The expense of this study was defrayed by a grant from the scientific Research Expenditure of the Department of Education, to which the author wishes to express his cordial thanks.

MATERIAL AND METHODS

The shrimp used for this work was *Paratya compressa* (DE HAAN) kept in the laboratory. The body weight of one shrimp was 0.255 g in the mean value. The animals were maintained in the large vessel for some days in the running water, providing with no food.

The apparatus employed for observing the changes in respiration rate will be indicated in the following Fig. 1. diagrammatically.

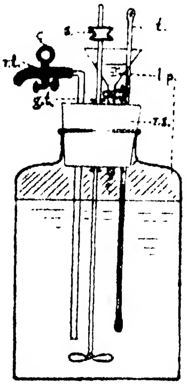

Fig. 1. The apparatus measuring the respiration.
c. clip, r.s. rubber stopper, r.t. rubber tube, g.t. glass tube, from which the sample water is drawn, t. thermometer, s. stirrer, l. p. liquid paraffin.

Ten animals were placed in the bottle which was filled with 500 cc water at first and closed with the liquid paraffin and rubber stopper. In this way, the oxygen consumption of the individuals was measured every 10 minutes, one minute of each interval being occupied by drawing off a sample and refilling the bottle with liquid paraffin.

The sample was drawn by the WINKLER's tube whose content was about 5 cc. Before taking the sample, the bottle was gently agitated for one minute by the stirrer to avoid stratification when the animals were resting on the bottom of the vessel. The shrimps remained in the bottle from the beginning to the end of one experiment. Water, solutions, and the bottle containing the animals were maintained at 22°C in a thermostat. The measuring apparatus for the respiration was set up in the dark room for photograph in the laboratory. When the illumination was in need, a 60 watt electic lamp was used on the outside of glass wall of the thermostat.

The oxygen content in the water was measured by the RISCH's micro-WINKLER method. The titration of the liberated iodine was performed with a 2 cc micro-burette graduated in 1/100 of 1 cc. The presence of copper salts introduces a slight error into the WINKLER method. At 0.01 N $CuSO_4$, this error will amount to about 2%, and with decrease in concentration it rapidly becomes inappreciable. The water employed for the determination of the normal and experimental respiration rates was the tap water in the laboratory saturated with air.

OBSERVATIONS AND EXPERIMENTS

1. *The Oxygen Consumption of the Shrimp under Light or Dark Conditions.*

The experiments for the normal respiration rate were divided into the following four series. The first one was under the condition of continuous illumination. The second one was under the condition of continuous darkness. The third series was under the illumination after the continuous darkness. The fouth series was under the darkness after the continuous illumination.

Table I

O_2-consumption cc./10 g at intervals (min.)	Conditions of the experiment			
	(I) Light adapted	(II) Dark adapted	(III) From dark to light	(IV) From light to dark
30′	4.06	3.86	3.12	3.30
60′	5.34	6.32	7.14	5.40
90′	8.60	8.07	11.31	8.62
120′	9.55	10.48	15.13	10.71

The results obtained from the experiment under these conditions were summarized in Table I. and Fig. 1. The oxygen consumption was given by the mean value of ten animals. Considering the diurnal rhythm in the respiration, the respiration rate was determined from 2.00 p.m. to 4.00 p.m. every day in September.

From this results it may be noticed that the respiration rate of the light phase transferred from the dark phase (III) is the maximum. But in the other three cases the considerable differences were not recognized.

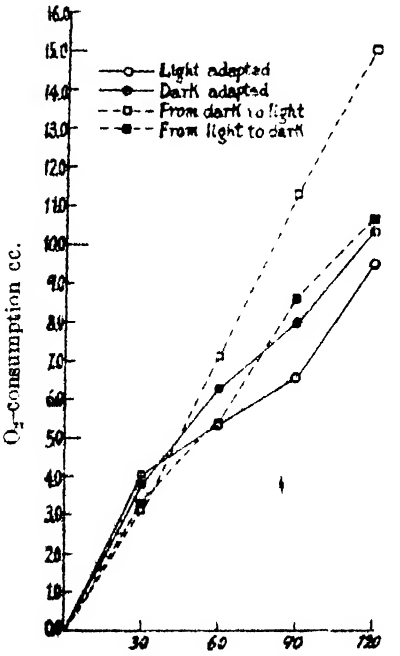

Fig. 2. O_2-consumption under light or dark conditions.

2. *The Effect of Oligodynamic Metals upon the Respiration Rate.*

For the heavy metals were used $CuSO_4$, $BaCl_2$ and $AgNO_3$ respectively. The measuring apparatus containing animals was illuminated by a 60 watt electric lamp in the

Table II. O_2-consumption affected by the various ionic heavy metals

Time in min.	Normal (control)	$CuSO_4$				$BaCl_2$		$HgCl_2$	$AgNO_3$
		0.008N	0.002N	0.0008 N	0.0004 N	0.006N	0.003N	0.00002 N	0.00002 N
10′	0.66	1.02	0.58	0.86	0.57	0.87	0.73	0.26	0.38
20′	1.09	1.82	1.25	1.35	0.96	1.51	1.11	0.96	0.97
30′	1.39	2.06	1.77	1.83	1.17	1.79	1.49	1.49	1.46
40′	2.05	2.53	1.97	2.02	/	2.23	2.11	2.09	1.69
50′	2.31	2.65	2.02	2.61	/	3.21	2.71	2.51	2.17
60′	3.36	3.04	3.02	2.77	1.76	3.72	3.25	2.57	2.64
90′	[illegible]	/	2.20	2.86	/	[illegible]	[illegible]	/	/
pH	7.1	6.5	5.7	6.2	6.0	[illegible]	6.3	6.6	6.6

course of this experiment. The representative results obtained are set out in Table II. and Fig. 2.

The conspicuous rise of respiration rate was observed in the animals immersed in the solution of copper sulphate, and there were also the remarkable expansion of the red pigment and rapid appearance of the blue pigment, which

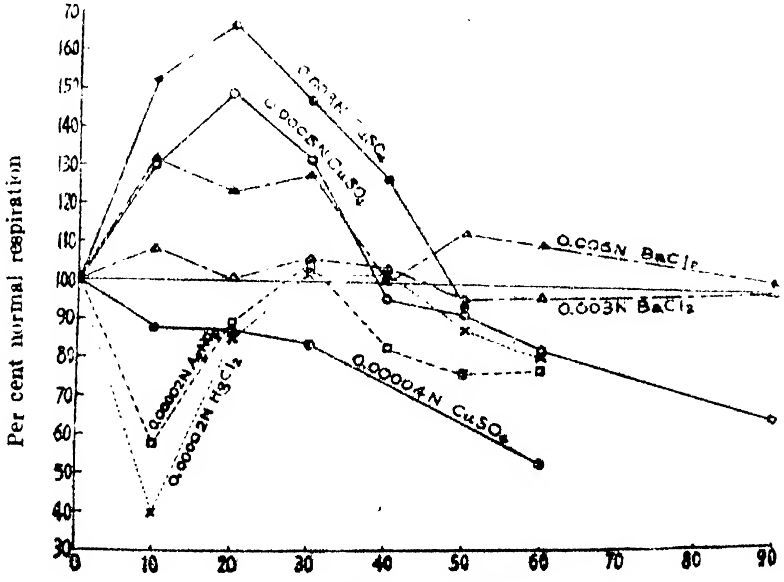

Fig. 3. Effect of ionic heavy metals on the oxygen consumption of the shrimp.

was described in my previous paper (NAGANO, 1943).

A similar rise in respiration rate was not seen with barium chloride in the similar concentration, although this salt acts as a powerful stimulant to muscular movement.

The experiment with $AgNO_3$ (0.0002N) and $HgCl_2$ (0.0002N) yielded the results essentially dissimilar to those obtained with copper sulphate. These salts inhibited the active muscular movement in that concentration and induced the disintegration of the animals.

3. *The Injection of the Copper Sulphate Solution into the Animal Body.*

The immersion of animal into the copper sulphate solution induced rapid appearance of the blue pigment surrounding the chromatophore. In this case, it will be noticed that the gill region was paralysed remarkably.

Then, the injection of the copper sulphate solution into the animal body was carried out. One dosis of the injection was 0.05 cc of 0.00004N $CuSO_4$. The red pigment expanded in 90 seconds to the maximum. The blue pigment appeared in 40 to 60 seconds. The former was able to expand also by the other heavy metalic solutions, but the latter only by the copper sulphate. The occurrence of the blue pigment could not be obtained by the injection of sulphates other than $CuSO_4$.

DISCUSSION AND CONCLUSION

In a brief review of the physiological effect of toxic copper solutions, MITCHELL (1938) concluded that the high toxicity of copper salts rests in the activity with which they combine with proteins, and that their penetration into living cells results in the precipitation of the cytoplasmic proteins as copper proteinates, and a gradual complete destruction of the entire protoplasmic structure.

Copper sulphate solutions are acid as a result of hydrolysis. Although the effect of hydrogen ions upon the respiration rate was not tested in the present work, it will be referred here the investigation about *Polycelis* by JONES (1941) in this point. That is to say, he states that solutions of pH 3.0—4.0 have a slight effect upon the respiration rate, but a pH of 5.0 has little action even in 24 hours. Since a pH of the most acid of copper sulphate solution used in my experiment with *Paratya* was 5.6, the hydrogen ion set free by hydrolysis seems not to be largely responsible for the respiration rate.

Whether the animals were kept in light or in dark for the long time (24 hours), there was no difference in the respiration rate. But the transference from the dark phase to light phase brought about the rapid rise of the respiration rate. Even if the behavior of the chromatophore in the light-or dark-adapted animals is related to metabolism in the whole body, we could not find the metabolic difference in light and dark phases by means of WINKLER's method. For this purpose, another exact method may be used; for example, the electrometric studies of oxidation-reduction system in the chromatophore.

The oligodynamic action of the heavy metals upon the respiration of the shrimp was remarkable. The copper sulphate accelerated the respiration at first in definite concentration, and the barium chloride excited it very slightly. But silver nitrate and mercuric chloride inhibited the respiration in the similar concentration.

GUTSTEIN (1932) has reached the conclusion that it is the phosphatid of the protoplasma which catches the heavy metals in the toxification of the cell. The toxic series for the various metals is as follows: Hg>Ag>Zn>Cu>Ba>Ca. In this case, the "phosphatids" will be probably the protein-phosphatid. It is very interesting that the oligodynamic action for the respiration rate in my experiment followed GUTSTEIN's series: Hg>Ag>Cu>Ba.

According to KEEBLE and GAMBLE (1910), the blue pigment (which is not granular) of the crustacean chromatophore is derived from the red one. An opinion confirmed by BROWN (1935) in *Palaemonetes*, however, regards the yellow pigment as the source of the blue coloration in *Leander adspersus*. In my previous observation of *Paratya compressa* (1943), was seen an evident disappearance of blue pigment in about two hours when the animal was placed on a white background in the laboratory. This fact can be most easily observed in shrimps taken out of the pond just before the experiment. In the present experiment, it was noticed that the occurrence of the blue pigment is related to the copper ion absorbed in the body of shrimps. But sulphate anion does not produce the blue pigment.

SUMMARY

1. The respiration rate of a shrimp *Paratya compressa* was remarkable when the dark-adapted animals were transferred to the constant illumination.

2. In a study on the effect of copper sulphate solution on the oxygen consumption of this shrimp, it was found that a definite concentration (0.008N～0.0008N) induced a marked preliminary rise in the respiration rate.

3. A slight increase of respiration was produced by barium chloride.

4. The rapid decline of the respiration rate was caused by silver nitrate and mercuric chloride.

5. The occurrence of the blue pigment surrounding the chromatophore may be related to the ionic copper metal absorbed in the body of shrimp.

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PHYSIOLOGICAL STUDIES ON THE PIGMENTARY SYSTEM OF CRUSTACEA. VII. THE EFFECT OF COLORED LIGHT ON PIGMENT MIGRATION OF THE COMPOUND EYE OF THE SHRIMPS*)**)

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(With 5 Text-figures)

INTRODUCTION

The vertebrates which have served in the study of pigment migration in the eye are fishes, doves, and especially frogs; while among the invertebrate moths, butterflies, *Daphnia* and crayfishes have mainly been examined. Between the years 1877, when BOLL (1877) discovered the pigment migration in the vertebrates and 1894, when KIESEL (1894) worked upon a moth *Plusia gamma*, the frog chiefly has served for the study of the effect of colored lights in this phenomenon.

In 1911, DAY showed that different amounts of pigment migration in the eye of crayfish were elicited by different regions of the spectrum at equal intensity. And he obtained the results that blue-violet was more efficient than red, as evidenced both by sections and by direct observations of crayfish eye.

Although a number of researches for the visual sensation about *Daphnia* and insects appeared recently, the most important investigation was MERKER's work (1929). He has pointed out the spectral limit to cause pigment migration of the compound eye of Lepidoptera (moths and butterflies), and other order of insect such as Hymenoptera (*Apis*) and Diptera (*Drosophila*). And he has proved that these insect eyes have the most highest sensitivity to the ultraviolet ray.

In particular, BERTHOLF (1933) has showed the exact method for determination of the distribution of stimulative efficiency in the spectral extent for *Drosophila*. YAGI (1941) has described the convenient method of determination of the sensitive wave length of spectrum for the pigment migration of the compoud eye of ***Chilo simplex***.

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*) This report is a part of the series of papers submitted to the Faculty of Science, Tôhoku University, in partial fulfilment of requirements of the degree of Doctor of Science, March 1948.

**) This study was financially aided by a grant from the Research Expenditure of the Department of Education, to which the author wishes to express his hearty thanks.

The present account covers somewhat the same ground, but undertakes to discover the most effective pigment migration in the shrimp eye and the author have investigated the nature of the pigment migration of the shrimp eye from various points of view. (Reports II, IV and V, 1947, 1948, 1950).

Before going further, the writer desires to express his appreciation to Prof. Dr. S. Nomura for his advice and guidance throughout the course of this work.

MATERIAL AND METHOD

The materials used for this investigation were two species of fresh water shrimps, *Paratya compressa* (de Haan) and *Leander paucidens* (de Haan), on which my previous experiments of the pigment migration have been attempted.

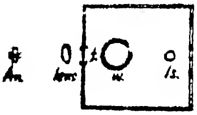

Fig: 1. Apparatus of the colored light's test.

An. animal
f. filter
w. heat absorbing water bottle
ls. light source

The apparatus for furnishing colored light was shown in Fig. 1. diagrammatically. The colore filters employed here were four kinds of purple = $V-P_1$ (400–450 mμ), blue = $V-B_1$ (467–500 mμ); green = $V-G_1$ (500–550 mμ), and red = $V-R_1$ (680–700 mμ) respectively. For the light source was used a 500 watt kinematographic electric lamp with spiral tungsten filament. By using this lamp were obtained wave lengths from 415 mμ to 700 mμ. The heat radiated from the light source was absorbed by water layer. The intensity of light was measure by the Matsuda's illuminometer which indicated directly the illuminating intensity as lux. By changing the distance from the light source to the eyes of the animal, the intensity on the object was regulated and the same intensity of 500 lux for all chromatic light was obtained.

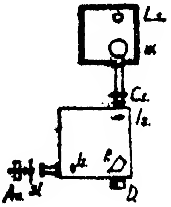

Fig. 2. Diagram showing arrangement of apparatus used in ascertaining for shrimp the stimulative effect of various spectral zone.

An. animal
sl. slit
ls. lens
D. wave length regulator
p. prism
Cs. collimeter slit
w. heat absorbing water bottle
L.s. light source

The previously dark adapted shrimps were fixed each on a slide glass sheeted with water containing cotton layer. The specimen was tied with a rubber tape slightly. Then the eye stalks of this animal were protruded from the edge of the slide glass to subject to the beam of colored light.

For the purpose of the test of effect of the monochromatized spectrum upon the eye, arrangements were made to project the bright spectrum on the scale by using Adam-Hilger's wave length spectometer (constant deviation type) (cf. Fig. 2). In this case, the illuminating intensity upon the eyes was only 10 lux.

As for the measurement of the degree of the retinal pigment migration, reference is made to the previous reports. (Report II, 1947; IV, 1948). The observation was done from October to November in the dark room of the laboratory, which was approximately at constant temperature (17° ± 1°C). The experiment was carried out definitely from 4.00 p. m. every day, considering the diurnal rhythmic movement of the eye pigment.

EXPERIMENT AND RESULTS

1. *The Pigment Migration in the Eye by Colored Light Using Colored Filters.*

The specimens were exposed to color light for 10, 20 and 30 minutes respectively. The migration index of the distal pigment in the eye was obtained from the mean value of five individuals in every experiment. The results of this experiment were plotted in Fig. 3.

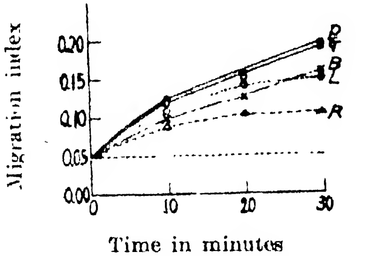

Fig. 3. The migration index of the distal pigment of the shrimp eye exposed in colored light.

(*Paratya compressa*)

P = purple light	— -●- —
G = green light	--□-
B = blue light	- -×- - -
R = red light	·· ···△ ·· ·
L = white light	······○······

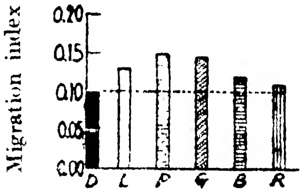

Fig. 4. The migration index.

D. . . . . . darkness
G. . . . . green light
L . . . . . . white light
B . . . . . blue light
P. . . . . . purple light
R. . . . . . red light

It may be mentioned that purple and green was most effective to the distal pigment migration in the eye of this fresh water shrimp. The white light produced the considerable effect on the eye pigment, but it was almost no effect after 20 minutes. The red light had the most weakest effect.

Next, in another fresh water shrimp, ***Leander paucidens***, the pigment migration affected by color light was measured in 10 minutes at the same intensity as the case of ***Paratya***. The result was represented in Fig. 4. In this case, it may be also said that purple and green is most effective and red is least effective to the distal eye pigment migration.

2. *The Determination of the most effective Wave Length of the Spectrum to the Eye Pigment Migration.*

From the result of the experiment by the colored filter, it was found that the effective light lies in the region between 500–550 mμ and 400–450 mμ respectively. Then, the most effective wave length of spectrum was searched for by the monochromatic light in visible part. In this case, the light intensity, however, was one fifteenth of that in the experiment with colored filter. Table I. shows the result with *Paratya*.

Table I

Wave length	Migration index in 15 min. (distal pigment)	Wave length	M.I. in 15 min. (distal pigment)
430	0.05	500	1.30
440	0.06	510	1.80
450	1.00	520	1.00
455	**1.80**	530	1.40
460	1.00	550	1.40

From this result, it may be inferred that the most effective wave length to the photomechanical action of the distal pigment in this shrimp eye is about 455 mμ and 510 mμ in the visible part of spectrum.

3. *The Behavior of the Retinal Pigment affected by Colored Light.*

The behavior of the eye pigments exposed to colored light was analysed by section. This result will be obviously seen in Fig. 5.

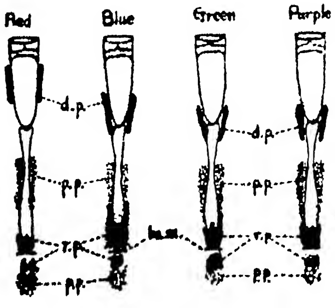

Fig. 5. The position of the retinal pigment affected by colored light.

d.p. ...... distal pigment.
p.p. ...... proximal pigment.
r.p. ...... reflecting pigment.
ba.m. ... basement membrane.

It was the most remarkable fact in this investigation that the reflecting pigment has remained above the basement membrane and the proximal pigment has migrated above the basement membrane as the light adapted eye.

DISCUSSION AND CONCLUSION

The results obtained show clearly that the behavior of the pigment in the shrimp eye is dependent upon the wave length of spectrum, certain colors are much more efficient as stimulating agents than other.

It may be interesting, briefly to compare the works of numerous other investigators in regard to the relative stimulative efficiency of light at different wave lengths for various animals in order to see where the results of the present investigation fit into the picture.

One of the first investigations in which both the wave length of light and the energy were measured was that by GROSS in 1913, in which he compared the stimulative effects of four different spectral zones of equal energy on several insects, *Drosophila*, *Feltia*, and *Calliphora* adults and *Zulzera* and *Calliphora* larvae. He found that for each of these the most effective wave lengths were in the blue-violet zone, between 430 and 490 mμ. MAST (1917) investigated the matter again for *Calliphora* larvae (and for a number of lower animals) in a carefully controlled experiment and found the most efficient energy for inhibiting, the stereotropism of *Tenebrio* larvae to be sharply localized in the neighborhood of 535 mμ. VISSCHER and LUCE (1928) compared the stimulative effect of 13 different spectral zones of equal energy on the cyprid larvae of the barnacle *Balanus* and found the region around 540 mμ most effective. The investigations of BERTHOLF (1931) on the honeybee indicate that the maximum in the visible spectrum for this animal is close to 553 mμ. Thus we see that the maximum for different animals even though fairly closely related is not the same. This has also been ably pointed out by HECHT (1928) and SCHLIEPER (1928). In my experiment it was concluded that the most effective wave lengths to the shrimp's eye pigment are in the neighborhood of 510 mμ and 455 mμ respectively.

SUMMARY

1. By measuring the migration index in distal pigment of the fresh water shrimps (*Paratya compressa* and *Leander paucidens*), the most efficient region in the spectrum to the eye pigments was determined.
2. The most effective wave lengths to the migration of the distal pigment of *Paratya*'s eye are in the neighborhood of 510 mμ (green) and 455 mμ (purple).
3. The proximal pigment of the shrimp's eye behaved to the colored light the same as to the lightness.

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STUDIES ON PHYSIOLOGY AND ECOLOGY OF PLANKTON
IV. TEMPERATURE COEFFICIENTS AND TEMPERATURE CHARACTERISTICS OF THE OXYGEN CONSUMPTION IN *SIMOCEPHALUS VETULUS*

By

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(With 1 Text-figure)

In the previous paper, the changes in the rate of oxygen consumption of the daphnid in course of the development were traced (HOSHI 1949 b), and it was found necessary for the purpose of physiological experiments to use the animals of the same developmental stage, because the numbers and developmental stages of the eggs or embryos in the brood chamber were not always the same even in brood-mothers originated from a single brood. The volume of oxygen consumed by a brood-mother is the total amount consumed by the mother herself and eggs or embryos developing in her brood chamber. For the purpose of minimizing the difference of respiratory rate due to the difference of developmental stages, the best way is to use the animals of the first instar stage.

The present paper deals with some data on the temperature coefficient (Q_{10}) and temperature characteristic (μ) of the oxygen consumption of the first instar young (released young) on the day they were discharged by the mother which had been cultured at 25°C. by the methods already reported (HOSHI 1949 a).

The animals used in the experiment measured 0.610 mm. in length, while those of the same stage used in experiments during the summer of 1949 measured 0.624 mm. in length (HOSHI 1950). Methods used for the measurement of respiration were the same as described previously. The experiment was carried out at eleven different temperatures between 30°C. and 8°C., from August to December 1947. In calculating the volume of oxygen, corrections were made for the water vapour tension and temperature. The respiratory values given in Table I are the averages of nine measurements.

The value of the temperature coefficient is 2.88 (15°C.-20°C.), 2.19 (20°C.-25°C.) and 1.99 (25°C.-30°C.) respectively; it becomes 1.18 between 8°C. and 15°C. In respect to the temperature coefficient for the oxygen consumption of the daphnid, there has been only one attempt which was presented by Obreshkove and Abramowitz (1932) in *Daphnia magna.* They reported that Q_{10} was 3.19 for the temperatures from 8°C. to 18°C. and was 1.73 between 18°C. and 28°C., subjecting the first brood-mother to the measurement.

Table I. Oxygen consumption of *Simocephalus vetulus*, first instar young, at various temperatures

Temperature (°C.)	8	10	13	15	18	20	23	25	26	(27)	28	30
O_2-consumption, mm³ × 10^{-3} per indiv. per hr.	37	38	39	42	49	70	84	104	109	(115)*	132	149
Q_{10}		1.18			2.88		2.19			1.99		

* This value was obtained during the summer of 1948, which is in accord with the data presented here (*cf.*, Hoshi 1950).

As in chemistry, the effect of temperature upon the velocity of various biological reactions can be expressed by Arrhenius' formula as follows:

$$K_2 = K_1 \cdot e^{\frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

where K_1 and K_2 are the velocity constants at the absolute temperatures, T_1 and T_2. To see whether this formula applies to the present data, the log rates of the oxygen consumption were plotted against the reciprocal of the absolute temperatures (Fig. 1).

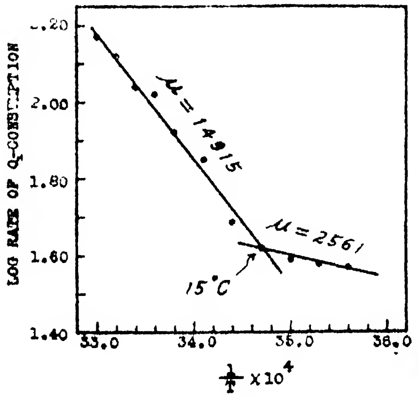

Fig. 1. The logarithm of the rate of oxygen consumption in *Simocephalus vetulus*, first instar young, plotted against the reciprocal of the absolute temperature.

The graph shows a distinct break at 15°C. and the slope of the line above this temperature gives the temperature characteristic μ of 14915. The value below 15°C. is 2561. The results of Obreshkove and Abramowitz (*loco cit.*) indicated that μ was 10017 in the temperature ranges from 18°C. to 31°C. and was 18923 from 8°C. to 18°C., which was found to be the critical temperature. In the present study 15°C. is the critical temperature, and 15°C. is one of the critical temperatures found most frequently in various biological phenomena, representing an occurrence of the "controlling reaction" (Crozier 1925).

In the present results, three Q_{10} values and one μ value in the higher temperature ranges correspond nearly to the values which have commonly found for the respiration in other various kinds of animals. Both Q_{10} and μ values in the lower ranges are much smaller than what have been reported by previous authors. The μ value of 14915 in this study and of 10017 obtained by OBRESHKOVE and ABRAMOWITZ, above the critical temperature, might be considered as a deviation from the value of 16100 and 11500 respectively, each of them being the temperature characteristic for oxidations (CROZIER, *loco cit.*).

There was no essential difference between the respiratory apparatus used by the present author and that of the authors above mentioned. In both cases a modification of THUNBERG's micro-respirometer was employed. And the μ value below the critical temperature in the present study is, as mentioned above, much smaller than those reported by them and also by other workers. This low value of μ below 15°C. may be connected, to some extent, with the fact that the animals used in this study were the descendants from the mothers which had been cultured at 25°C. throughout the course of experiments, as stated previously.

BĚLEHRÁDEK (1926) proposed an empirical formula for the relation between temperature and velocity. His formula runs as follows:

$$y = \frac{a}{t^b}, \quad \text{or} \quad \log y = \log a - b \cdot \log t$$

where y = time, t = temperature; a and b are constants. On application of this formula to the present data, the constant b, which is regarded as a temperature coefficient, was found to be 1.04 between 8°C. and 30°C. No linear relationship was obtained in this case between *log* temperature and *log* time. It has been reported that the value of b generally lies between 1.0 and 3.0 (BĚLEHRÁDEK 1935). As regards this constant, however, HEILBRUNN (1938) states that "in view of empirical nature of the equation, the constant b has no physical or biological significance."

The author wishes here to express his gratitude to Prof. SHICHIROKU NOMURA for kind suggestions.

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STUDIES ON PHYSIOLOGY AND ECOLOGY OF PLANKTON V. FATTY SUBSTANCE IN DEVELOPMENT OF *SIMOCEPHALUS VETULUS*, WITH REFERENCE TO BEHAVIOR OF YOLK GRANULE

By

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(With 1 Text-figure)

The summer egg of *Simocephalus* deposited in the brood chamber is compactly filled with a plenty of yolk granules, and in the centre of the egg a large oil drop is found. The size of the yolk granules in earlier stage is, at largest, ten μ in diameter. Some of these yolk granules, then, fuse gradually into a large yolk mass, the size of which extends from thirty to forty μ in diameter. This fusion of yolk granules takes place from gastrula, and all of the yolk masses are gathered around the oil drop at the time of the bursting of the egg-membrane. The yolk granules in a yolk mass are isolated from one another at the beginning, but in the later stage they tend to melt together into uniform phase. In course of the absorption of the yolk material the mass becomes small and irregular in form.

The oil drop, which is found singly in the centre of the egg, is divided into a few numbers of small droplets in the later stage. Besides the oil drop, some number of oil droplets are found among the yolk granules in the egg, but they never appear in the yolk granule of earlier stage. These drops and droplets are, in later stage, taken in the yolk masses. But the formers are found isolated from the latter in the yolk mass. With advance of development, the gradual decrease of oil substance results in the remainder of minute quantities of the oil droplets on the dorsal side of the body in the free-swimming stage (released young).

On the other hand, the oil drops and droplets become somewhat reddish gradually in the later stage while the drop in newly deposited egg is almost colorless. Therefore, some changes in quality as well as in quantity in the oil drop and the droplets would be perceivable in course of consumption of oil substance.

The aim of this paper is to describe what kind of substance is utilized especially in the earlier development of *Simocephalus vetulus* (O. F. Müller).

In the earlier stage, the yolk granules stored compactly in the egg prevent us from clear observation of the oil drop and droplets. The treatment with centrifuge of the egg enabled us easily to observe an exact aspect of the oil drop. The centrifugal force used in the experiment was 1058 times gravity. The egg-membrane withstands the same centrifugal force without any rupture even for one hour. The results are shown as follows:

a) *Gastrula.*

The oil drop migrates to the contripetal side, and the droplets are found around it (Fig. 1, A). The yolk granules, on the other hand, run to the centrifugal side. Between the oil drop and the yolk granules is situated the cytoplasm. Among the yolk granules, the smaller ones are situated on the centrifugal side. The larger yolk granules, which seem to contain much more carotinoid pigment than the smaller ones, are found near the cytoplasm. This pigment, in later stages, disappears nearly from the yolk mass and diffuses in various tissues of the body.

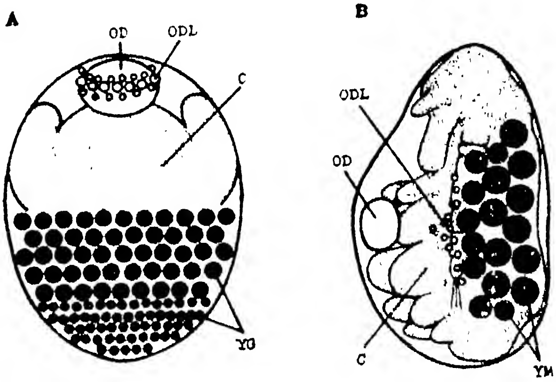

Fig. 1. Diagrammatic representation of embryo treated with centrifuge.

A Gastrula.
B Nauplius.

OD, oil drop; ODL, oil droplet; YG, yolk granule; YM, yolk mass; C, cytoplasm.

b) *Nauplius.*

In the nauplius, as well as in gastrula, the oil drop migrates also to the centripetal side (Fig. 1, B). The oil droplets in this stage do not appear around the oil drop but are situated among the cytoplasm or remain near the yolk masses. On the centrifugal side are found the yolk masses. In many cases it was observed that the oil drop was situated on the ventral side of the body while the yolk masses remained on the dorsal side. This is interesting from the fact that the absorption of the yolk material is larger or more rapid on the ventral side than on the dorsal.

c) *Hatched Embryo and Released Young.*

In these stages, no perceivable effect of centrifugation was found in the body. The oil drops and the yolk masses, both of which now having become small, do not migrate but remain almost unmovd. It might be considered that the arrangement of various tissues formed in the body might prevent their migration.

The embryos subjected to centrifugation were, then, stained vitally with fat-dyes: Sudan III, Scharlach rot and Nile blue. The oil drop and droplets were stained brilliant yellowish orange with the former two. With the latter they were colored brilliant pink. It is obvious, therefore, that these drops and droplets contain a kind of fatty substance. Judging from the color of the oil drops stained, we can consider some glycerin-ester as their chief content. As mentioned above, this fatty substance decreases gradually with advance of development and the small amount of the droplets remains only on the dorsal side of the body in the free-swimming stage. In other words, the gradual decrease of the fatty substance existed in the oil drop suggests the utilization of fat during the embryonic development.

As to the energy source of the embryos, we pointed out in the previous paper, from the results of studies on the changes in respiratory quotient during development, that fat was mainly utilized in the gastrula (HOSHI 1950). The present study, therefore, adds a datum which supports the concept that during earlier stages of development fat plays the principal rôle of the energy supply.

The results of KONOPACKA (1924) and also of BRACHET (1934), which indicated the utilization of fat in earlier stages of amphibian embryos, call here our attention in connection with the energy source of the embryonic development.

Recently ÖHMAN (1940, '45) reported results similar to the present study in connection with the fat-utilization in the early stage of sea-urchin embryos.

The author wishes here to express his hearty thanks to Prof. SHICHIROKU NOMURA for kind suggestions and criticisms in this study.

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STUDIES IN THE ASSOCIATIVE ECOLOGY OF INSECTS

I. NOCTURNAL SUCCESSION OF A MOSQUITO ASSOCIATION IN THE BITING ACTIVITY*

By

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(With 4 Text-figures)

There are many reports concerning the nocturnal or diurnal activity of mosquitoes (Kumm and Novis, 1938 ; Mitsui, 1942 ; Bates, 1944 ; Ishiguro, 1944 ; Haddow, 1945, ; Hasegawa, 1944, 1945 ; Yoshida and others, 1947). However, investigations from the synecological view point are scanty. The day and night succession of the sand dune insect community was studied by Chapman and others (1926), but they made no quantitative treatments. In the studies in nocturnal ecology, Park and others (1931) observed the nocturnal activity of animals in relation to the environmental conditions, but their studies on the associative analysis was insufficient. In the present paper, the writers describe the nocturnal succession of the mosquito association in the biting activity.

The writers are indebted to Drs. Isao Motomura, Gunji Tomita and Yasuo Abe for their criticisms and advices.

EXPERIMENTS

The experiments were done at Funaoka near Sendai, from August 27th to September 1st, 1946. A mosquito-net (1 m^3) was set at the entrance of a stable and the hourly catches of mosquitoes were done.

RESULTS AND DISCUSSION

During the course of the experiments, the following six species of mosquitoes were obtained: ***Armigeres obturbans, Aedes vexans nipponii, Mansonia uniformis, Anopheles hyrcanus sinensis, Culex tritaeniorhynchus, Culex pipiens pallens.***

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* The expense of this work was partly defrayed by a grant from the Scientific Research Expenditure of the Department of Education.

I. *Biting Cycle of the Mosquitoes.*

As is expected in the insects whose activities are governed by photic environment, numerous mosquitoes enter the stable at the evening dusk, early in the night and then at the morning twilight (Fig. 1). It is very interesting to notice that there is the specific photic reaction, that is the specificity in the optimal range of illumination intensity which influences the activity, and thus in the evening and early in the night the activity becomes vigorous in the order of *Armigeres*, *Aedes*, *Mansonia*, *Anopheles* and *Culex*, but is reversed at the morning.

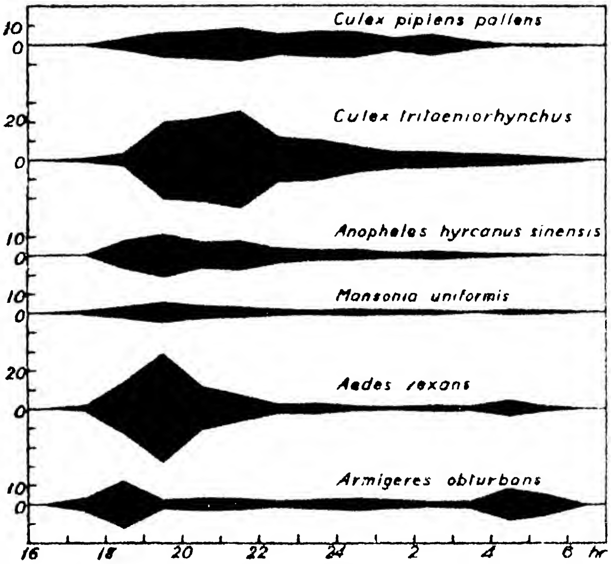

Fig. 1. Hourly sequence of the individual number of each species in the nocturnal course of activity.

II. *Succession in the Mosquito Association.*

a. *The Form of Association.*

As above mentioned, each species of mosquitoes has characteristic flight habit. Now the mosquitoes entering into the net should be accepted as one type of the so-called animal association. MOTOMURA (1932) instituted a formula, $\log y + ax = b$, where x is the rank in individual number of each species of which an association consists, y is the number of its individuals and a and b are constants. By plotting the logarithms of y against x we should expect a straight line, from the slope of it, the value of a can be obtained. Thus the value of a should express whether the association is simple or complex. Here the writers should like to notice that the value of b is accepted as the index of the population density of the association. Therefore the form of an association is expressed by using the values of *a* and *b*.

In the present investigation, it was tested whether or not the every hour's catches can be explained by Motomura's formula. In the successive hourly catches, a linear distribution of the logarithms of individual number of every species against their ranks in number was obtained without exception, probability of non-linearity being always more than 20 per cent.

As is shown in Figure 2, the values of *a* and *b* are not always equal. At the 17-18th hr. the value of *a* is fairly large, that is the composition of the catch is rather simple,. *Armigeres* being the dominant species. But at the 18-19th hr. the value of *a* decreases remarkably to the level of 0.20, namely the composition becomes suddenly complex. The values of *b* are rather small in the evening dusk, but thereafter increases markedly and thus the high population continues till 21th hr. During the latter half of night the small values of *a* and *b* run with parallel, that is to say the activity becomes very weak. The value of *a* markedly falls at the 3-4th hr. to about 0.12, where there are no so-called dominant species, and the population density is thinest, being small the value of *b*. The composition becomes at the 4-5th hr. suddenly simple, *Aedes* being the dominant species.

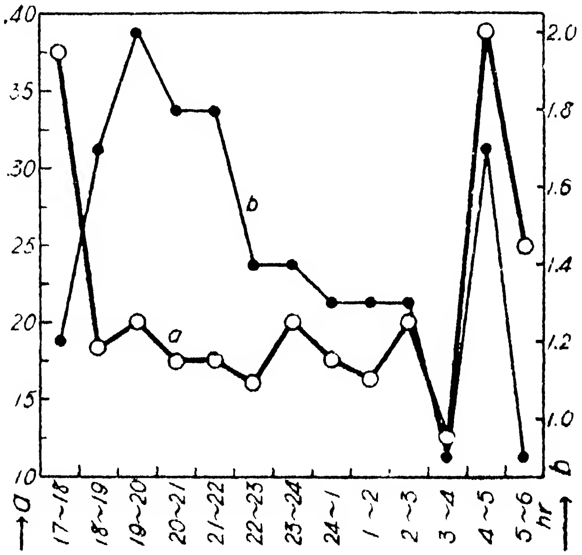

Fig. 2. Showing the change in the form of hourly catches by the value of *a* and *b*.

Conclusively the mosquito association is simple at the evening dusk where *Aedes* is dominant, very complex in the night with *Culex* as the representative species, and again becomes simple in the morning twilight when *Aedes* is dominant.

b. *Succession in the Mosquito Association.*

To determine the change in the composion of the hourly catches in the nocturnal course of biting activity, the correlation method given by Motomura (1935) was applied.

Between the individual number of each species in an hour's catch and that of the corresponding species in the following hour, the coefficient of correlation was calculated, and the results thus obtained are shown in Figure 3, where white spots show non-significancy and black ones significancy.

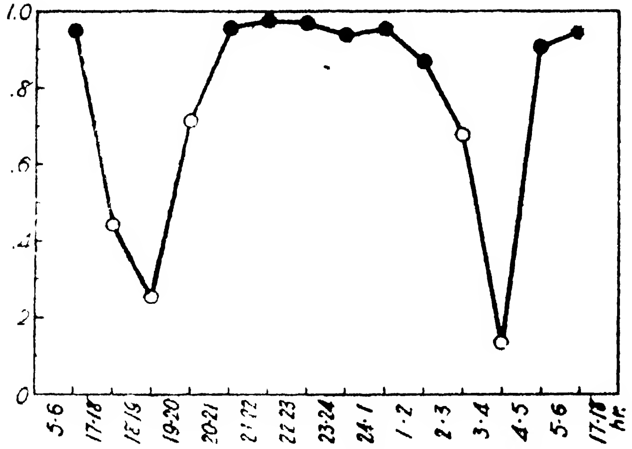

Fig. 3. Showing the relation between hourly catches by the coefficients of correlation.

Until the 20th hr. the correlation coefficients are fairly low, therefore the components of the hourly catches in the evening and early in the night may be expected to change one after another. But after the 20th hr. the hourly catches correlate with each other very highly, the coefficients of correlation being 0. 9 or so. It may thus be conceivable that the composition of catches in these hours do not change, in other words the association is very stable. But after the 3–4th hr. the composition changes suddenly and shows a very low correlation, being only 0. 14 between the 3-4th hr and the 4–5th hr. In the morning the correlation again becomes very high.

Therefore, it may be concluded that the unstable mosquito association in the dusk extends into the stable night association and is followed again by the unstable one in the morning twilight.

Since it was desirable to know more definitely the succession of association, the reciprocal relation between the hourly catches was investigated by extending the above mentioned correlation method (Fig. 4). Thus, 13 series of correlation coefficients were obtained, from which we were able to classify the mosquito association into several consocia. As is clear in Figure 4, the first three series are independent of each other, so that the mosquito association should change every one hour. The series from the 4th to 7th resemble each other in their characters and the same can be said for the following three series, namely from the 8th to 10th. Thus, three consocia are recognized in the night. The association changes suddenly from the 11th series into the remarkably different 12th series, which resemble the 13th, so we may here expect two consocia.

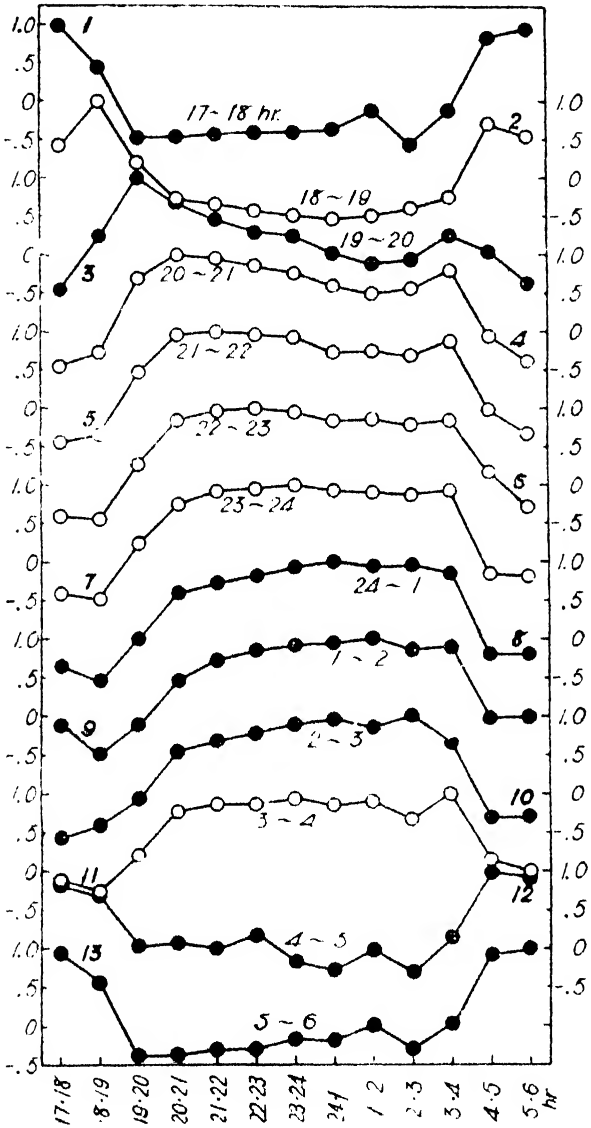

Fig. 4. 13 series of coefficients of correlation obtained by the reciprocal treatment of hourly catches.

Seven series, those from the 4th to 7th and from the 8th to 10th, belong to the stable phase and the first three series in the evening and last three in the morning are referable to the unstable phase. It is very interesting to know that the first series resembles the 13th series.

Finally we can summarize the succestion in mosquito association in the biting activity into the following system of three phases and seven consocia.

I. *Unstable Phase in the Evening*

a. *Armigeres obturbans*-consocium ; b. *Aedes vexans=Armigeres obturbans*-consocium ; c. *Aedes vexans*-consocium.

II. *Stable Phase in the Night*

d. *Culex tritaeniorhynchus*-consocium ; e. *Culex trit.=Culex pipiens pallens*-consocium.

III. *Unstable Phase in the Morning*

f. Phase of weakened activity ; g. *Armigeres obturbans*-consocium.

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STUDIES IN THE ASSOCIATIVE ECOLOGY OF INSECTS
II. SYNECOLOGICAL INVESTIGATION OF THE LARVAL HABITATS OF MOSQUITOES*

By

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(With 2 Text-figures)

The breeding places and larval habitats of various mosquitoes hitherto reported contain but brief descriptions without details of investigation (Nomura, 1943; Hosoi, 1948; Bates, 1949; Shogaki, 1950). The writers, to know additional light on the problem, here deal with the synecological investigation of the larval habitats of various mosquitoes in Sendai.

The writers are obliged to Drs. Isao Motomura, Genji Tomita and Yasuo Abe for their criticisms.

RESULTS OF SURVEY

The larval survey of 1946 resulted in finding from various water bodies in Sendai the following 18 species of mosquitoes; ***Aedes togoi***, ***Aedes japonicus***, ***Aedes flavopictus***, ***Aedes vexans nipponii***, ***Aedes esoensis***, ***Aedes niveus***, ***Armigeres obturbans***, ***Tripteroides bambusa***, ***Anopheles hyrcanus sinensis***, ***Anopheles sineroides***, ***Anopheles lindesaii japonicus***, ***Anopheles koreicus***, ***Culex pipiens pallens***, ***Culex tritaeniorhynchus***, ***Culex vorax***, ***Culex hayashii***, ***Culex orientalis*** and ***Culex bitaeniorhynchus***. The water bodies were classified empirically into ground pool, spring, pond, swamp, paddy field, bamboo flower vase of graveyard, stone flower vase of graveyard, stone basin, water barrel, foul water, cess pool in farm, remnant of toilet pool and tree hole.

In the present investigation are recorded only the water bodies where mosquito larvae were found, and the density of the larval population was not researched.

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* The expense of this work was partly defrayed by a grant from the Scientific Research Expenditure of the Department of Education.

RESULTS OF INVESTIGATION

Applying the correlation method (MOTOMURA, 1935, KATÔ and TORIUMI, 1950), two problems were quantitatively dealt with, viz. the kind of species in relation to water situation, and the kind of larval association in relation to water body.

I. *Larval Association.*

In this study 10 species were used as the other 8 were too rarely for statistical handling. The correlation coefficient between the frequency for a certain species collected from various water situations and for the other species from corresponding water bodies was calculated successively, and thus 10 series of correlation coefficients for the 10 species were obtained, from which the mosquitoes could be classified into several groups (Fig. 1.).

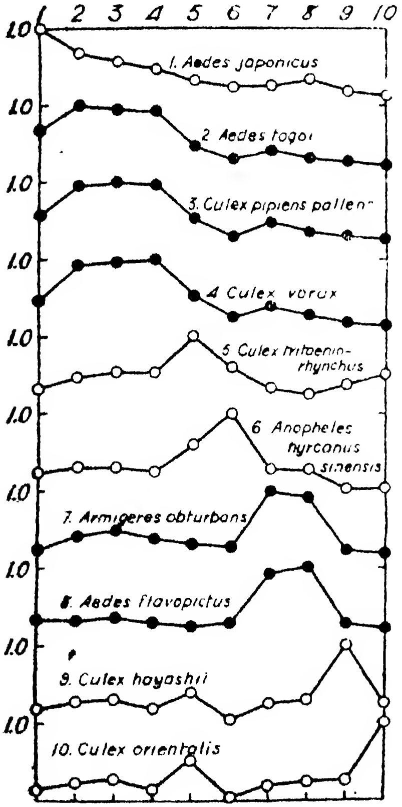

Fig. 1. 10 series of coefficients of correlation obtained by the reciprocal treatment of 10 species of mosquittoes. Figures on abscissa correspond respectively No. of species.

1. The first series of *Aedes japonicus* is independent to all other series, but slightly resembles the following three series in characters. Judging from the fact that these three species, *Aedes togoi*, *Culex pipiens pallens* and *Culex vorax*, have each other similar character in the series of correlation coefficients, they may be found in similar aquatic situations, where *Aedes japonicus* occassionally grows.

2. The character of the 5th series, *Culex tritaeniorhynchus*, and of the 6th, *Anopheles sinensis*, resemble each other, but the correlation coefficient between these two series is not so large, that the breeding places of these two species may not always be similar.

3. *Armigeres obturbans* and *Aedes flavopictus* must grow in similar water situations, for the 7th series closely resemble the 8th in its character.

4. The correlation between *Culex hayashii*-series and *Culex orientalis*-series is fairly low, due to habitat, the former in the spring, and the latter in the paddy field.

II. *Larval Habitats.*

The writers dealt with 12 water situations, except the tree hole where *Aedes flavopictus* was found only once. From the frequency obtained for various mosquito larvae in a certain water body and for the corresponding species in other water bodies, the correlation coefficients were successively calculated, and the reciprocal relation between 12 water situations is represented by 12 coefficient-series; these are divided into six groups (Fig. 2).

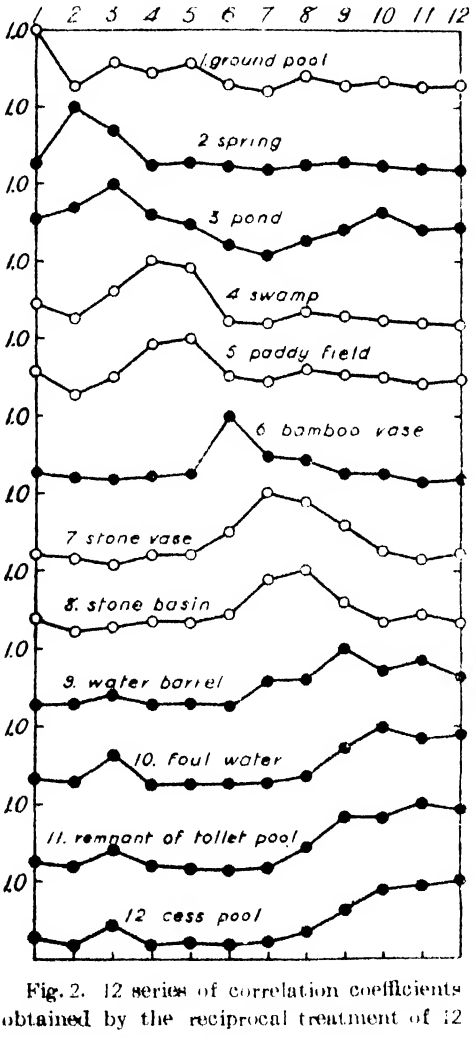

Fig. 2. 12 series of correlation coefficients obtained by the reciprocal treatment of 12 water situations. Figures on abscissa correspond respectively No. of water bodies

1. The ground pool including various small water areas on the ground, where various mosquitoes, for instance, *Aedes vexans*, *Anopheles sineroides*, *Culex hayashii* and occasionally *Culex tritaeniorhynchus* are found, is independent of any other water bodies.

2. From the fact that the 2nd series fairly well resembles the 3rd, it is conceivable that a similar mosquito association may be found in these two situations, spring and pond, where *Culex hayashii* and *Anopheles sineroides* are the representative species.

3 The swamp- and paddy field-series are similar in their characters, where *Anopheles* association is found mixing with *Culex tritaeniorhynchus*.

4. The bamboo vase of graveyard is a peculiar situation for the mosquito larvae, forming *Aedes flavopictus-Armigeres obturbans*=association, and *Tripteroides bambusa* is here occasionally found.

5. It is very interesting that the charcters of coefficient series of the stone vase and of stone basin quite differ from that of the bamboo vase, in spite of that these three situations are in the graveyard. Here the simple association

composed mainly of *Aedes japonicus* is found, and occasionally ***Aedes togoi*** and rarely ***Aedes flavopictus*** and ***Culex tritaeniorhynchus*** also breed.

6. Judging from the characters of coefficient series, water barrel, foul water, remnant of toilet pool and cess pool are similar in their environment for the mosquito breeding, forming *Aedes togoi-Culex pipiens pallens-Culex vorax*=association. Attention should be given to that the water barrel-series resembles somewhat the stone vase and stone basin, due to the occassional mixing of *Aedes japonicus*.

CONCLUSION

The larval habitats of various mosquitoes found near Sendai were synecologically investigated by means of the correlation method.

1. It seems that various mosqitoes tend to form the following associations, that is to say, the following five groups of mosquito larvae grow respectively in similar breeding places.

a. *Aedes japonicus*; b. *Aedes togoi-Culex vorax* (-*Aedes japonicus*);
c. *Anopheles hyrcanus sinensis-Culex tritaeniorhynchus* (-*Culex orientalis*);
d. *Armigeres obturbans-Aedes flavopictus*;
e. *Culex hayashii-Anopheles sineroides*.

2. Various water situations can be divided into the following six groups, in each of which a characteristic mosquito association may be formed.

a. Foul water- remnant of toilet pool- cess pool- water barrel;
b. Stone vase- stone basin in graveyard; c. Bamboo vase of graveyard;
d. Swamp-paddy field; e. Pond-spring; f. Ground pool.

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ECOLOGICAL NOTE OF THE SPAWNING CYCLE OF THE SCALLOP, *PECTEN YESSOENSIS* JAY, IN MUTSU BAY[1)2)]

By

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(With 1 Text-figure)

I. FLUCTUATION OF THE NUMBER OF LARVAE AND SPATS

The differences in the annual catches of the scallop were reported by KISHINOUYE (1896), NISHIOKA (1943), and SATÔ and SASAKI (1945). The latter concluded that the differences were due to the changes in the period of appearance of the young.

The present writer studied the differences in the number of swimming larvae and spats of the bivalve from the season of 1941 to 1950 in Mutsu Bay; the number of swimming larvae in the years 1942, '46, '48, '49 and '50 are respectively 5, 786, 3096, 38 and 556 per cubic meter of sea water caught by means of vertical net collections, and in the other years no larva was found. The number of spats in the years 1942, '43, '46, '48, '49 and '50 are respectively 1/20, 1/30, 9, 76, 1/3 and 5 per area of 100 sq. cm. of collectors, and in the other years no spat was caught (Fig. 1).

II. INDUCTION OF SPAWNING OF THE SCALLOP

It is reported by KINOSHITA (1943) that the scallop is induced to spawn by a 5°C. rise in temperature and 0.4 rise in pH of the medium. The writer (YAMAMOTO, 1949, a) found in the laboratory that the low effective temperature of the spawning is 8.0–8.5°C., and the spawning is inactivated in lower temperatures, and on the contrary is stimulated by a sudden rise in temperature, even by 0.5°C. above the effective temperature, and also that the falling of pH inhibits the spwaning, but no effect was seen with the rising of it. Such stimulation is more effective during the high breeding season.

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1) Contribution from the Marine Biological Station, Asamushi, Aomori-Ken, No. 183.

2) Aided by a grant from the Scientific Research Expenditure of the Department of Education.

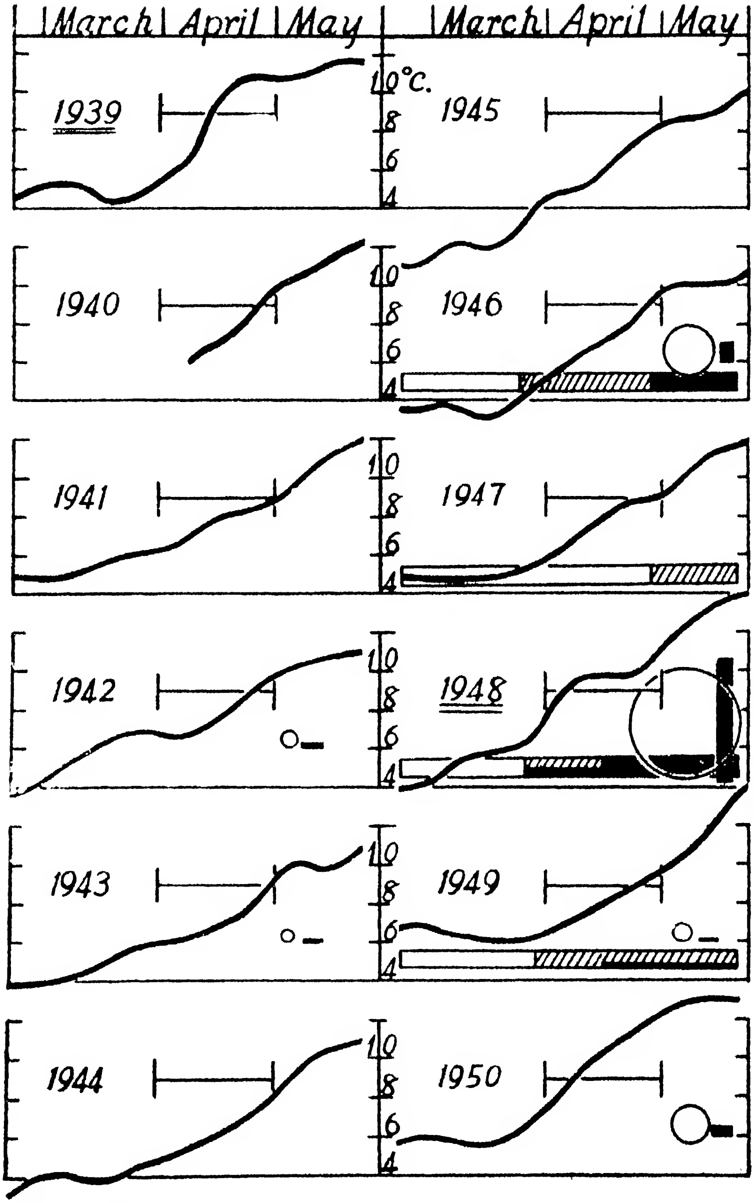

Fig. 1. Smoothed curves of coast temperature averaged for five consecutive days. Horizontal lines in April represent the level of the low effective temperature for spawning and the period of high breeding season. Size of circles and vertical bars show respectively the relative amounts of swimming larvae and attached spats, and the years with underlines indicate that spats were found abundantly. Horizontal bands at the bottom of graphs show the characters of dominant species of plankton, *viz.* white, hatched and black parts represent respectively characters of cold, moderate and warm water.

It is already reported and generally accepted that temperature is the most important factor controlling the spawning of many lamellibranchs (NELSON, 1928a, *etc.*), especially in *Ostrea virginica* (GALTSOFF, 1938, *etc.*) under natural conditions.

The water temperature of the coast (Fig. 1) runs parallel with that of the bottom, and thus 8.0–8.5°C., which is the effective temperature of spawning, is represented by 9.0–9.2°C. of the coast temperature.

III. BREEDING SEASON

The gonad of the female scallop in Mutsu Bay in December contains primary oöcyte which is in resting period during the winter months. Thereafter, the oöcyte in the condition of metaphase of the first maturation division, *viz.*, the ripe ovum is spawned and fertilized by spermatozoon in the sea water (YAMAMOTO, 1943).

The high breeding season continues always to late April, and the ovarian eggs degenerate at the beginning of May, and it seems that some of them are absorbed and others are discharged. The visceral mass containing the ovary, therefore, shrinks early in May. Such degeneration of the gonads has been found both under natural and laboratory conditions with or without spawning, and has also been recognized in various animals, especially in lamellibranchs, *viz. Pecten opercularis*, *P. latiauritus*, *Venus mercenaria*, *Ostrea virginica*, *O. edulis* (AMIRTHALINGAM, 1928; COE, '45; GALTSOFF, 38a; LOOSANOFF, '37, '42; ORTON, '27).

Most scallops, about 60%, contain the ripe ova from the beginning to the end of April, and then suddenly the ripe individuals decrease in number to 5–10% at the beginning of May. It is, therefore, possible to induce the spawning in large quantity only during the period from the beginning to the end of April, and only in a small percentage at the beginning of May.

Thus, it is concluded that the high breeding season of the animal is seen in April in Mutsu Bay and is not governed largely by the environmental conditions but rather seems to be due to the physiological sexual cycle.

IV. GENERAL CONSIDERATIONS

The years when the sea water temperature reached the effective temperature of spawning in April are as follows: 1939, '40, '42, '43, '46, '47, '48, '49 and '50 (Fig. 1). Judging from these facts it is believed that the scallop was able to spawn only in these years, in which period the swimming larvae and attached spats were collected; no spawning was observed in the other years. Basing upon this fact it is conceivable that the changes in appearance of the larvae and spats in Mutsu Bay run parallel with the fluctuation of spawning of the scallop.

Moreover, in 1939, 1946 and 1948, the temperature curves leaped suddenly above the effective temperature level, and in these three years, the swimming larvae and spats were abundantly found as already described. Apparently such discontinuous sudden rising of sea water temperature, as in the experiments of spawning induction in laboratory tanks, would induce the spawning of the scallop also under natural conditions. On the contrary, the slow rise of the temperature may not, to any great extent, affect the spawning, for the sudden rising of temperature alone seems to stimulate the spawning reaction. The case of 1949 reveals the above mentioned conditions (Fig. 1).

The leaping of the temperature above-mentioned was affected by the Tsushima warm current, a branch of the Japan stream. Fluctuation of the warm current flowing into the bay has been investigated by KOKUBO (from 1946 onward) who says that the succession of the dominant plankton species represents more precisely the conditions of sea water, and in spring characteristic diatoms of *Chaetoceros socialis* and *C. debilis* are replaced by *C. affinis*, an indicator of moderate water, and then later by *C. decipiens* of the warm water type. In 1946 and '48 the succession was more remarkable and especially in 1948, *C. decipiens* appeared dominantly already at the beginning of April, and the temperature leaped above the critical temperature in the same period. Further, in the late April of 1946, the sea water of the open sea, which was affected by the warm current, flowed into the bay and raised the sea water temperature higher than that of the same season of any other years observed.

Generally saying, the ascending of the sea water temperature by the air temperature and solar radiation is more striking near the coast, and, on the contrary, more remarkably affected off shore by flowing oceanic warm water. The discontinuous sudden rising, therefore, is expected to be more remarkable off shore than that shown in Fig. 1.

Finally, the spawning of the scallop in Mutsu Bay is perhaps induced by the sudden rising of the water temperature due to the strong warm water current, which flows into the bay during the breeding season, and without such phenomena the gonads of the scallop probably degenerate and the visceral masses shrink. The similar fact has been reported in *O. virginica* (NELSON, 1928; HOPKINS, '31). The slow ascending of temperature delays the reaction period for spawning of the oyster and induces no remarkable spawning (NELSON, 1928).

Finally the writer wishes to express his obligations to Profs. I. MOTOMURA and M. KATÔ for their helpful suggestions.

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BENTHIC COMMUNITIES IN MUTSU BAY1)2)

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(With 3 Text-figures)

COMPOSITION OF BENTHOS

1. The composition of benthos collected by EKMAN-BIRGE's bottom sampler with opening of 1/44 sq. m. at 24 stations in Mutsu Bay is as follows: polychaetous annelids 57.8%, molluscs 25.2%, echinoderms 6.7%, crustaceans 2.8% and others 7.5%. The composition of benthos is not uniform throughout the bay, but varies with the stations.

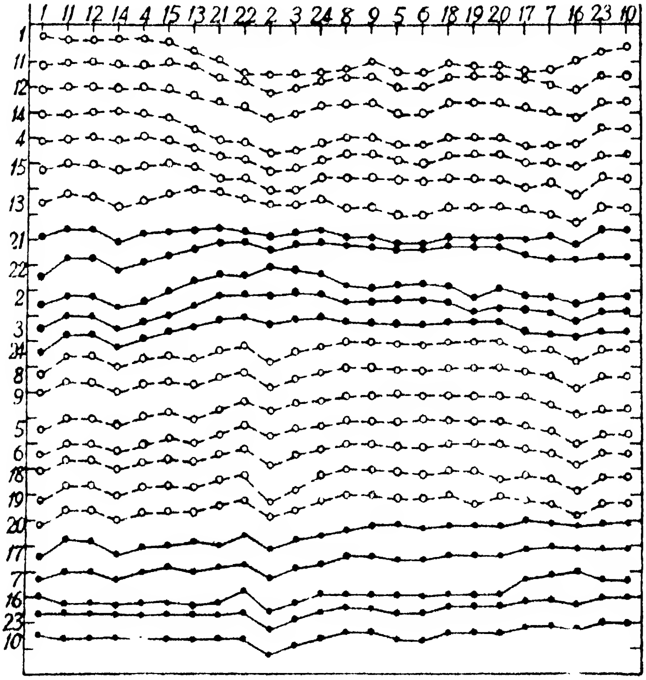

Fig. 1. Reciprocal ralation between the compositions of benthos obtained from 24 stations represented by correlation coefficients.

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1) Contribution from the Marine Biological Station, Asamushi, Aomori-Ken, No. 184.

2) Aided by a grant from the Scientific Research Expenditure of the Department of Education.

According to MOTOMURA (1935), the correlation coefficient between the composition of each station was calculated one by one and thus the reciprocal relation between each station was represented by 24 series of correlation coefficients (Fig. 1). Here, the correlation between the stations 15, 13 and 21; 24 and 8; 20 and 17 are statistically insignificant and therefore they are divided into four groups or four benthic communities which can be distinguished in Mutsu Bay (Fig. 2a).

2. In order to clarify the forms of these four communities, the application of MOTOMURA's law of geometrical progression (MOTOMURA, 1932), $log y + ax = b$ where y is the number of individuals of one species and x is its rank in number, was tested. As is shown in Fig. 3 the law is applicable to the benthic communities of Mutsu Bay with 95% reliability.

The value of a, which indicates whether the community is simple or complex, is so small in the first and fourth communities that it is recognized that these two are complex in their composition, and on the contrary, the benthos is fairly simple in the second and third communities, the value of a being fairly large.

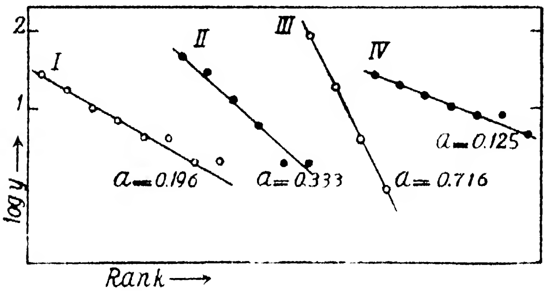


Fig. 3. Applying the law of geometrical progression.

3. The first benthic community, which occupies the mouth of the bay is dominated by scaphopods amounting to 22%, most of which are ***Dentalium*** sp., followed by a polychaetous annelid in 14%, and a crustacean amphipod in 10%. It was clearly shown by the value a that the composition is so complex, and it is rather difficult to explain the character of the community with only one or two representative species. The bottom material of the ground consists of sandy mud.

The second community is fairly simple. Scaphopods, most of whom are also ***Dentalium*** sp., are dominant making 53% of the composition in this area, followed by the polychaetous annelid ***Telepsavus*** in 24%, and the annelid ***Maldane sarsi*** in 12%. The substratum consists of fine mud, occupying the center of Aomori Bay.

The third community is the most simple one. The community is dominated by the polychaetous annelid ***Maldane*** in 80% of composition, and another in 15%, while the ophiuroid ***Ophiophragmus japonicus*** was found only in 2%. This community is characterized with the dominant species, ***Maldane sarsi***, and is clearly distinguishable from the second community. The bottom is formed of fine mud in the east half of the center of Mutsu Bay and in the innermost of Aomori Bay.

In the fourth community, the ophiuroidean echinoderm *Ophiura sarsii* is found in 22% and is the dominant species, followed by one species of polychaetous annelid in 17%, an echinoid *Echinocardium* in 14%, and a pelecypod *Pecten yessoensis* is in 0.7%, being the twelfth rank in composition. Although this ground occupies the coastal parts of the bay, it is more extensive in the east than in the western part of the bay. The substratum consists of mud with coarse sand and gravels.

Conclusively there are four benthic communities in Mutsu Bay; a complex Scaphopoda-Polychaeta-Amphipoda community at the mouth of the bay, a simple Scaphopoda community in the central region of Aomori Bay, a simple Polychaeta community in the east half and also in the innermost of Aomori Bay, and a complex Ophiura-Polychaeta-Echinocardium community of the coast region.

NUMERICAL AND GRAVIMETRIC POPULATION

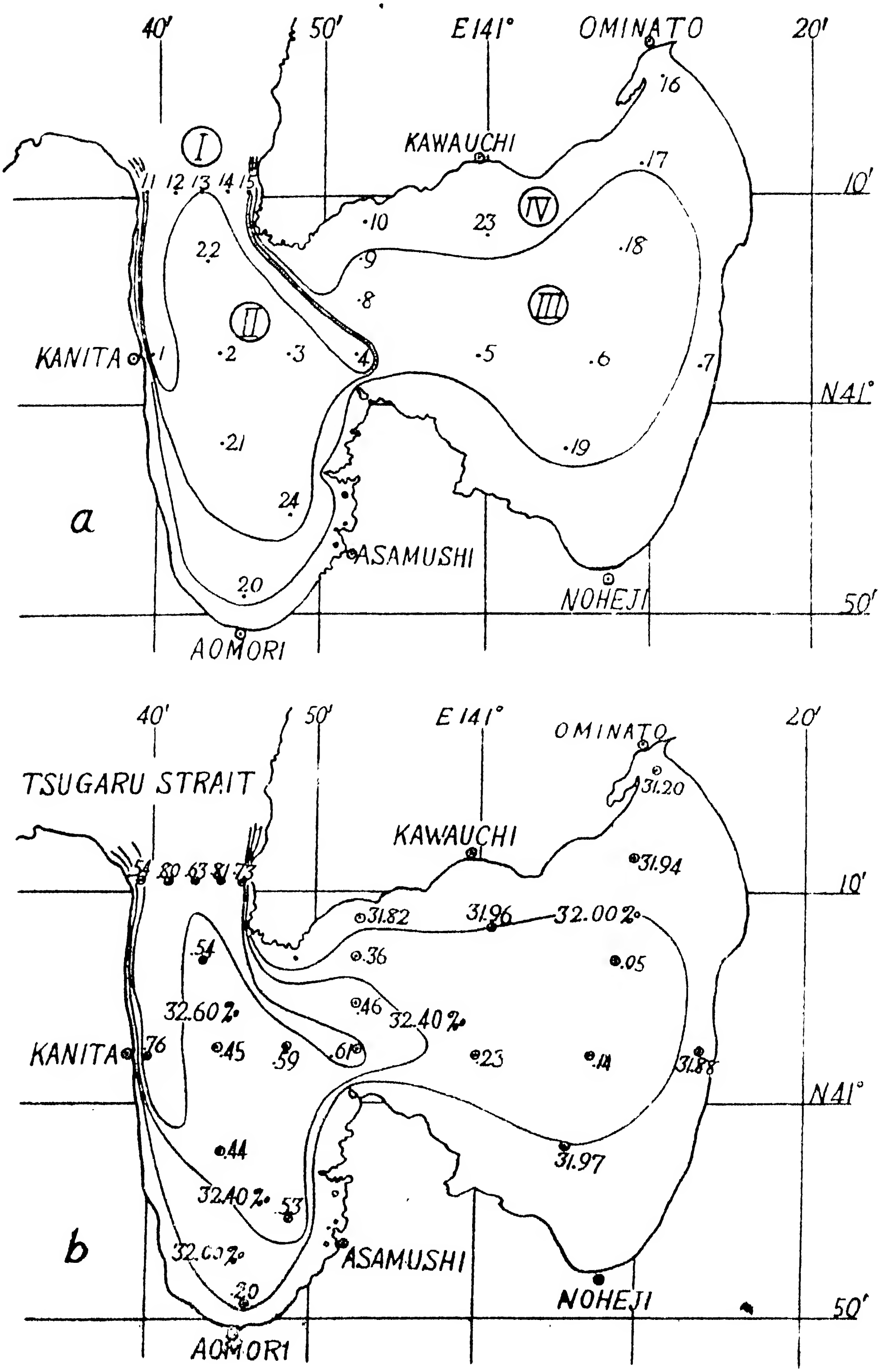
Of the whole bay, the numerical and gravimetric population is 330 per square meter and 175.1 g. per square meter respectively in the spring and in the summer of 1947 (Table I). In the same season of 1948, 368 and 113.8 g. per square meter respectively. The value obtained at each station, however, fluctuates considerably.

Table I. Numerical and gravimetric productivities of benthos in 1947 in Mutsu Bay

	Including animals with hard shell		Excluding animals with hard shell	
	Number of animals per sq. m.	Weight in grams per sq. m.	Number of animals per sq. m.	Weight in grams per sq. m.
Bay as a whole	330.0	175.1	225.3	126.7
Ground I	237.6	116.2	74.8	45.3
Ground II	427.2	60.7	238.9	56.3
Ground III	466.4	236.3	462.0	235.0
Ground IV	198.0	279.0	132.0	168.1

Fig. 2, a. Map showing the 24 stations sampled and the area of the four benthic communities divided by means of applying the correlation method.

b. Map showing isohaline curves of bottom layer indicated by mean value obtained in May to August in 1947 and 1948.


40'
50'
E 141°
OMINATO
20'
KAWAUCHI
10'
N 41°
50'
KANITA
ASAMUSHI
NOHEJI
AOMORI
I
II
III
IV
a
11 12 13 14 15
1 2 3 4 5 6 7 8 9 10
16 17 18 19 20 21 22 23 24
b
TSUGARU STRAIT
31.20
31.94
31.82
31.96
32.00‰
.05
.36
.46
32.40‰
.54
32.60‰
.76
.45
.59
.61
.23
.14
31.88
.44
31.97
32.40‰
.53
32.00‰
.20
.54 .80 .63 .81 .73

This shows that the benthic animals do not distribute uniformly in the bay, and differ in the four grounds and even in the same grounds. The fact that the value obtained in both years is apparently equal seems to indicate the stability of population density in the whole and even in each community.

It is noteworthy in the fourth community (Table I) that the gravimetric productivity reaches a high value notwithstanding the minimum value in the numerical population. Although the numerical population density comes to the medium in omparison with that of other bays (MIYADI, 1940a, etc.), the gravimetric population is remarkably great, according mostly to the results obtained in the third and fourth grounds, especially in the latter.

GENERAL CONSIDERATIONS

It is one of the characteristics of the benthic communities of Mutsu Bay that the composition of polychaetous annelids reaches more than half of the total composition, and therefore, judging from the previous papers (MIYADI, 1940a, etc.), this bay seems to be an oligotrophic one. Although the composition of the benthos collected in the bay are different in each station, the already mentioned dominant animals in each of the four communities may be taken as an indicator of the oligotrophic bay; the view is supported by the results obtained in many Japanese bays (MIYADI, 1940a, etc.).

On the other hand, the gravimetric population of Mutsu Bay is greater than the usual oligotrophic Japanese bays, and is rather comparable to that of many north European coasts (PETERSEN & BOYSEN JENSEN, 1911; PETERSEN, 1918; SPÄRCK, 1929, '35), and also of the Japanese eutrophic bays as Nanao Bay, Tôkyô Bay, Kasaoka Bay, and Matsunaga Bay. This fact seems to be characterized by the area of the third and fourth communities of the bay. As was reported by many authors concerning the benthos and plankton in coasts and lakes (IDELSON, 1934; SPÄRCK, 1935; MIYADI, 1932a, etc.; KOKUBO, 1940), the fact that the large weight shown in Mutsu Bay seems to be one character of the bay in high latitude.

There are many reports on the factors controlling the productivity in the bay and the configuration of benthic communities (BROCH, 1927, etc.; CASPER, 1922; DREW, 1906; KINOSHITA *et al*, 1944a, etc.; MIYADI, 1944; NISHIOKA & YAMAMOTO, 1943; SHELFORD, 1916, 1925). It is, however, very interesting that the distribution of salinity of the bottom layer, which is represented by the mean value obtained six times in 1947 and '48, agrees well with the configuration of the benthic communities of the bay.

It is known that the accumulation of effect of environmental conditions, such as the mixture of oceanic and coast waters, is indicated by salinity. Thus it is naturally expected as above mentioned that the benthic configuration depends upon the salinity of the bottom layer.

Finally I wish to express my thanks to Prof. Dr. M. KATÔ for his valuable advice and criticisms, and to Mr. T. HANAOKA, the Director of the Fisheries Institute in the Inland Sea for his encouragement. Further I wish to acknowledge my obligation to Dr. K. HATAI, Mr. K. ÔYAMA, and Mr. S. MURAKAMI for the identification of animals, and to Mr. T. ETO for his kind assistance in field work.

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BOTANICAL STUDIES OF BOG LAKES IN A VOLCANIC REGION WITH SPECIAL REFERENCE TO LACUSTRINE BACTERIA[1]

PART IV. DISCUSSION

By

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In my previous papers of this series (JIMBÔ, 1949, 1950) I have described what I have seen from different angles with regard to Naga-Numa, an exceedingly interesting bog lake lying in the volcanic region of Mt. Hakkôda. A great many things that we have learnt therefrom may convey an idea of what bog lakes in our country are like. Among them I have to stress above all the development of a bog round a water basin fed by an acid seepage and the consequent dystrophication of the basin itself, which acid, sulphuric acid in this case, is, in all probability, linked with the volcanic action. That is to say, lakes of that sort owe their acidity not merely to the organic acid of the humus materials but also to the mineral acid originally present in the seepage. I think I am justified in saying that bog lakes of such an origin closely connected with the volcanic action occur very commonly, if not invariably, elsewhere in our country studded with a vast number of volcanoes.

I would like now to add some experimental results and discuss certain issues of importance. Eschewing to repeat discussion to any great extent as to what have been dealt with at some length in the first two parts of the present series of papers, let us turn now to the microbiological aspects of this bog lake.

At the outset of this series of articles I alluded to the status of the lake bacteriology with which we are now faced. It is a pity that there are no available data with regard to the microbial world in the lakes of Japan in advance of mine, except for the brief bacterial counts made of Takasuka-Numa (SUGAWARA, 1935) and Suwa-Ko (HÔGETSU, 1948), both classed in the category of eutrophic lakes. As a consequence, we are almost unable to compare the findings of my own regarding this one with others. All the same, brief mention will be made of certain points of great importance emerging from a general survey of the mass of foreign papers, which, in part, are undoubtedly also true of this lake or have a considerable bearing on it.

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[1] Contribution from the Mt. Hakkôda Botanical Laboratory. No. 35.

Apart from the lag of research in our country, counts have been made of the bacterial populations of some lakes principally in the United States and Europe. The trends of the horizontal and vertical distributions of bacteria as well as the seasonal changes in these respects in the course of years were pursued. On the other hand, the presence of particular groups of bacteria was confirmed. A good many subjects have been touched, but, so far, I have come across none of these which have ever been dealt with deeply or exhaustively. It is not my intention to deal with all these points any further. For the broad outline of our present knowledge of lake bacteria the reader is referred to HENRICI (1939), who has also traced the historical aspects and cited a body of articles concerned.

Autochthonous Lacustrine Bacteria. Whether these are specific bacteria confined to the lacustrine habitat is to be ranked among the most fundamental questions of the lake bacteriology. As yet these problems remain unsolved. To-day, however, most of workers incline to believe the presence of a body of autochthonous bacteria characteristic of waters, apart from spirilla, and iron and sulphur bacteria akin to some extent to algae. Thus, SNOW and FRED (1926) and HENRICI (1939) pointed out some facts which suggest the presence of autochthonous bacteria thriving at relatively low temperatures.

A lake receives all the time an enormous number of bacteria from the air as well as from inlets and surface drainage. As to what becomes of these bacteria in the long run, there remains the question: Are these allochthonous bacteria able to maintain themselves therein, or sooner or later to die away without adding to the bacterial flora of the lake? In this connection, the following experiments were conducted to test for the presence of any substance in the water of Naga-Numa which exerts an inhibitory effect on the growth of common soil bacteria and fungi. I compared two series of cultures of soil bacteria and fungi, one with media made up of the ordinary water and the other with those containing a decoction of the bottom mud of Naga-Numa instead of the common water. The mud extract was neutralized prior to the use. Soil from a fertile field was plated out on the sodium-caseinate agar and WAKSMAN's acid medium with and without the mud extract. After an appropriate period of incubation any difference could hardly be discernible between the two series of plates with both qualitative and quantitative respects. Moreover, the same was true of enrichment cultures of *Azotobacter*, *Clostridium*, nitrifying bacteria and cellulose-decomposing organisms in the soil sample. It will be seen from this fact that, as far as thermostable constituents are concerned, the water of this dystrophic lake does not contain something detrimental to the soil microorganisms. The sea water, on the other hand, is known to inhibit the growth of most nonmarine bacteria. Lately ROSENFELD and ZOBELL (1947) noticed the production of antibiotic substances by some of marine bacteria and ventured to suggest that the bactericidal property of sea water is due largely to the content of antibiotics of that sort, neither the salinity nor the high osmotic value alone being capable of accounting for it.

Occurrence of Physiological Groups of Bacteria. As for several physiological groups of bacteria playing a vital role in the cycles of essential elements, many attempts have been made by earlier workers to obtain selective cultures. So far as I can make out, however, they are confined to eu- and oligotrophic lakes, containing no references to dystrophic ones. Denitrifying bacteria, whose presence both in the water and in the bottom mud of Naga-Numa was proved, are probably widespread in a variety of lakes of every type (*vide* KLEIN and STEINER, 1929; ALLGEIER, PETERSON, JUDAY and BIRGE, 1932; GRAHAM and YOUNG, 1934; KUSNETZOW, 1934).

Bacterial Number. Whilst plate counts have been made of bacteria in the water of a number of lakes in America and Europe, there is no record concerning dystrophic lakes. Apart from the strikingly high values of two eutrophic lakes in our country mentioned above—from 175 to 43,500 of bacteria per c.c. of the water of Takasuka-Numa and as many as from thousands to hundred millions of bacteria in Suwa-Ko, the bacterial number determined repeatedly in Lake Mendota, an eutrophic lake hitherto investigated at greater length from different angles, ranges between 25 and 40,000—for the most part from 100 to 3,000 (FRED, WILSON and DAVENPORT, 1924; SNOW and FRED, 1926; DOMOGALLA, FRED and PETERSON, 1926). In the oligotrophic Flathead Lake the bacterial population is one-tenth as many as in Lake Mendota (GRAHAM and YOUNG, 1934). The records in Grosser Plöner See (BAIL, 1903), Balatonsee (ZIH, 1929), Lunzer Seen (ZIH, 1932) and Rotsee (DUEGGELI, 1934) are all within these ranges. The value of Naga-Numa (close to 1,000) is admittedly higher than those of the oligotrophic lakes and is comparable with the medium value of the eutrophic ones.

As to the plate counts of the bottom mud, HENRICI and McCOY (1938) who worked with Lake Alexander (Minnesota), Lake Mendota and other Wisconsin lakes recorded from thousands to hundred millions—usually from ten thousands to millions—of bacteria per c.c. of the surface layer of mud. On the whole, the more productive the lake, the higher is the bacterial content of the mud. Dystrophic lakes outside the series of eu- and oligotrophic lakes are comparatively rich in bacteria. Thus the surface muds of Helmet Lake and Mary Lake contain from 87,000 to 270,000 and from 68,000 to 101,000 bacteria per c.c. respectively. In Europe, on the other hand, were counted from 1,000 to 2,500 bacteria per c.c. both in Balatonsee and in Lunzer Seen (ZIH, 1929, 1932) and about 1,000,000 in

Rotsee (Dueggeli, 1936). The above figures of two dystrophic lakes in Wisconsin are thought to be more or less comparable with that of Naga-Numa, when assuming the water content of the muds to be little short of 90 per cent.

Benthic Bacteria and their Environment. As has already been emphasized, the bottom mud of Naga-Numa is practically free from chromogenic bacteria which are plentiful in the water. But according to Allgeier, Peterson, Juday and Birge (1932), as much as 10 per cent. of the total number of bacteria are pigmented—mostly yellow—in the mud of Lake Mendota that is eutrophic. Henrici and McCoy (1938) confirmed this and found that the proportion of pigmented bacteria falls off with the depth from 17 per cent. at the mud surface to 10 per cent. at a depth of 18 cm., below which there is a marked reduction. The latter authors stated, however, that the muds of dystrophic lakes are strikingly poor in chromogenic bacteria. Although oxygen disappears from the bottom water of Lake Mendota only temporarily during the summer stagnation period, its bottom ought to be provided with oxygen to some extent. Whether the proportion of chromogenic bacteria in the bottom mud reflects clearly the environmental conditions in the bottom remains to see.

I would like to note here that, although the bacterial counts taken in Naga-Numa may contain an insignificant number of actinomycetes which were hardly discernible in the plates, the actinomycetes are altogether excluded from the number of pigmented bacteria hitherto given.

It is worth while to quote in connection with the faculty for absorbing oxygen of the *dy* the work of Miyadi (1934), who worked with the bottom deposits of a number of Japanese lakes ranked among eutrophic and oligotrophic lakes of every kind. While, as a rule, the higher the degree of eutrophication the more is absorbed oxygen, a notable exception to it is Lake Suigetsu, which is characterized by the permanently stagnant hypolimnion (consequently free from oxygen and containing hydrogen sulphide), epilimnion being markedly eutrophic (*vide also* Jimbô, 1938). The black mud on its bottom can absorb only a very small amount of oxygen. This is likely to be due to the absence of aerobes very largely responsible for the oxygen absorption. This fact seems in support of the view put forward by Loennerblad (1930) that the oxygen absorption by *gyttja* is to be regarded as dependent mainly upon the microbial activity unlike that by *dy* brought about chemically for the most part.

I would like to add to what has been mentioned in regard to the benthic fauna inhabiting Naga-Numa, that *Chaoborus* larvae are also found beside the *Chironomus* larvae already referred to in the preceding papers.

Fungus Population. It has already been mentioned in a preceding paper that the fungus population of the bottom mud of Naga-Numa is strikingly distinctive, characterized by the predominance of two particular species and the paucity of the other species, whereas the pool water and the air over the pool contain much the same fungus flora relatively rich in species without one or more particular fungi being dominant. In this connection I will describe shortly the similar results obtained recently in regard to the fungus population of Lake Biwa, which will be detailed elsewhere.

On a recent visit to the Ôtsu Hydrobiological Station I was enabled by courtesy of the Director Dr M. Ueno to carry out a brief survey of the fungus flora of the lake. In the middle of September of 1949 the surface water and the superficial layer of the bottom deposit were taken at a station over 1 km. off the Station and some 4 m. deep and plated out on Waksman's acid medium. Furthermore a batch of sterile plates filled with this medium were exposed to the atmosphere on the lake when sampling. 5 fungi per c.c. of the water and some 170 per gram of the fresh mud were counted. For certain this oligotrophic lake is a little less heavily polluted by fungi than the dystrophic one on Mt. Hakkôda. Though the *Mortierella* characteristic of the mud of the latter is lacking, a blue-green species of *Penicillium* and a species of *Trichoderma*, instead of the *Mortierella*, are predominating in the mud of Lake Biwa as well, each of which represents at times even about one in three of all the fungus colonies developed. The fungus flora of the mud is also distinct from those of the water and the overlying air just as in the case of Naga-Numa. It is worth noting that, while the dominant forms in the mud of Naga-Numa are a mortierella and a penicillium, those of Lake Biwa are a trichoderma and a penicillium. The question arises as to whether the species of the main benthic fungi are largely dependent on the lake type and, in consequence, may be regarded as an index of the productivity. It remains to be a theme of future study.

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PLANT COMMUNITIES DEVELOPED IN THE PLACE OF SNOW-PATCHES ON MT. HAKKÔDA

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(With 2 Text-figures)

INTRODUCTION

On high mountains with abundant snowfall, snow remains until late summer in the form of snow-patches in valleys and depressions or on the leeward slope of the prevailing winter winds. As already stressed by Hayashi (1935), the plants thus covered with snow-patches are suddenly exposed by the rapid snow melt to the summer heat, and they are forced to grow during a short time. In places where snow-patches remain too late to permit development of normal vegetation, there flourish peculiar plant communities dominated by some alpine dwarf shrubs, grasses, herbs, mosses and liverworts, which are capable of surviving and establishing themselves under such extreme conditions. Most of these "snow-patch communities", as they are called, are considered to have been arrested in their normal development up to the culminative stage, due mainly to the effect of the local abundance of snow accumulation, which is largely related to the physiography. From this point of view, they may be regarded as physiographic subclimaxes.

Attention was called to the plant communities characteristic of the snow-patch by a number of ecologists, dating from Wahlenberg (*see* Schröter 1926, p. 641), who first described those in the Alps. They have worked along this line in the Alps, Scandinavian and Scottish mountains, and the Rocky Mountains in the United States. Brockmann-Jerosch (1907), Braun-Blanquet (1913), Braun-Blanquet *et al.* (1932), Fries (1913), Frey (1922), Schröter (1926), Harshberger (1929) and Gjaerevoll (1950) have treated them from sociolgical viewpoints. Among papers dealing with autoecological problems of snow-patches, that by Rompel (1928) is notable.

According to Schröter (1926), the snow-patch communities (*Schneetälchenrasen*) of the Alps are restricted to the slopes higher than the middle part of the alpine zone (above 2500 m. in altitude at Puschlav). On Mt. Hakkôda, however, they are seen even in the lower part of the subalpine zone (1000 m.) surrounded by a typical subalpine coniferous forest of Abietetum Mariesii. The characteristic

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1) Contribution from the Mt. Hakkôda Botanical Laboratory, No. 36.

snow-patch communities of this mountain are covered with snow presumably about one month longer than in the Alps. For example, BRAUN-BLANQUET *et al.* (1932) describes that Salicetum herbaceae, a typical snow-patch association in the Alps which occupies the outer part of the snow-patches, requires an exposure of 2½ to 4 months for its growth, while Polytrichetum, a common moss community in the inner parts of the European snow-patches, flourishes in places of less than three months' exposure. On Mt. Hakkôda, as described herein, the typical snow-patch communities are restricted to places which are exposed for less than three months, and the moss community (*Kiaeria falcata* sociation) to places with only 1/2 to 1 month's exposure. These differences may possibly be dependent upon the fact that, on this mountain, snow lies more deeply and snow-patches last longer than in the Alps at the same zone of vertical distribution of plant communities.

Little is known in Japan of the relationships between snow and vegetation, except for some notes by Prof. YOSHII and others. Thus, HAYASHI (1935) worked on the development of snow-patch plants on Mt. Hakkôda in the course of thawing affected by the striking changes in air and soil temperatures and light intensity. YOSHII (1948, 1949) pointed out the marked effect of snow on vegetation, especially on the notable deformation of trees at high altitudes.

The present paper gives the results of my synecological observations made on Mt. Hakkôda during my stay in the Mt. Hakkôda Botanical Laboratory in two consecutive summers of 1948 and 1949.

TOPOGRAPHY IN RELATION TO SNOW ACCUMULATION

Mt. Hakkôda (1585 m. above sea-level, 40° 35′ N.) is located at the northern part of Honshû and during winter it is usually covered with four to five metres of snow. The thickness of the snow cover varies considerably according to both topography and direction of prevailing winds. In North Japan strong west or north-west winds prevail during the winter months, as is the case with the eastern coast of North America. As a consequence, on this mountain, the snow is deep particularly on the lee slopes facing the east and south-east. The leeward slopes of the peaks of Mt. Hakkôda are almost completely covered with snow till early June, whereas the other slopes are then only sporadically spotted with snow. Towards the beginning of July, the snow-patches are already confined to the former slopes. Some of the snow-patches remain till the middle of September, and in years of heavy snowfall, they persist even until early October when snow falls anew. Thus, the lee slope may, in part, be bare of snow for four months or thereabout, i. e. from early June onwards, whilst the interval between the disappearance of the last snow-patch and the new snowfall is generally only half a month. On the other slopes, it is likely that a considerable area is uncovered for no less than four months. The longer the length of time for which snow lies the more marked is the effect upon the development of plant communities after snow has melted, and, in reality, prominent snow-patch communities are noticed where the ground is snow-free for less than three months. The enormous accumulation of snow on the leeward slope which is responsible for the snow-patches remaining until late summer may possibly be due to the circumstance that the adjoining windward slope is gentle enough to hold the fallen snow that will be subsequently drifted upon the lee slope, and that the latter slope itself has a sufficient inclination to be sheltered from the wind.

EFFECTS OF SNOW ON THE FOREST LIMIT AND ON THE ALPINE SHRUB COMMUNITIES

The effect of remaining snow is prominently displayed in the altitude of the forest limit and the composition of the alpine shrub communities above it. The

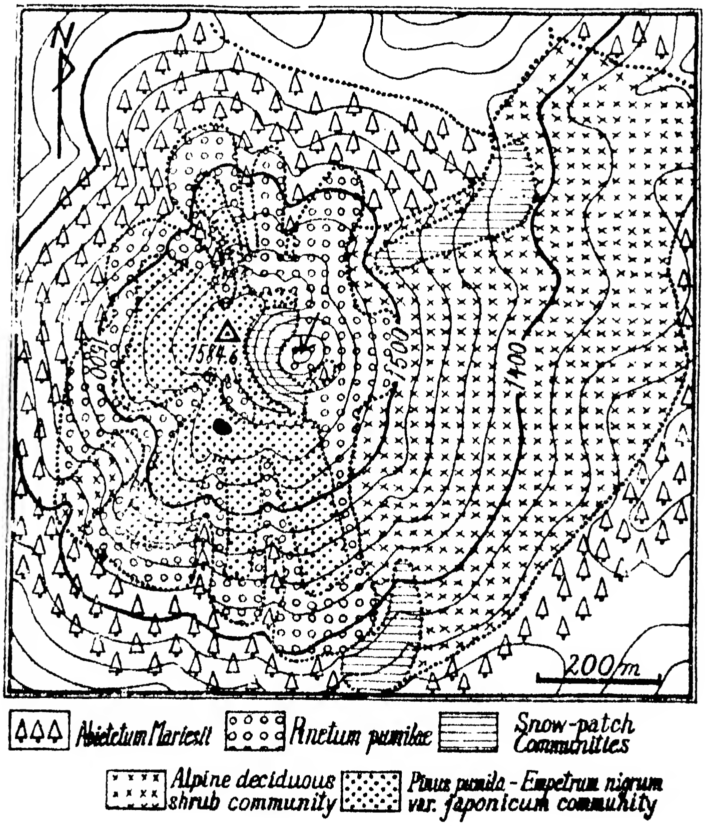
N
1584.6
1500
1400
200m
Abietetum Mariesii
Alnetum pumilae
Snow-patch Communities
Alpine deciduous shrub community
Pinus pumila - Empetrum nigrum var. japonicum community

Fig. 1. Vegetation map of Ōdake, the highest peak of Mt. Hakkōda.

forest limit of Mt. Hakkôda is at the upper limit of Abietetum Mariesii, a sub-alpine coniferous forest, which is adjoined above by alpine shrub communities. That of Ôdake, the highest of several peaks of Mt. Hakkôda, lies on the leeward slope rich in snow some 200 m. lower than on the other slopes, due, in all probability, to the limited growth of *Abies Mariesii* owing to the late snow melt (Table I, Figs. 1 and 2).

Table I. The altitude (m. above sea-level) of the forest limit on Ôdake in different exposures

Exposure	N	NNE	E	ESE	SE	SSE	SSW	SW	WNW	NNW
Altitude	1470	1465	1320	1320	1340	1390	1410	1460	1510	1480

The alpine shrub communities of this mountain fall into three types, that is to say, two of dwarf pines—Pinetum pumilae and *Pinus pumila-Empetrum nigrum* var. *japonicum* community—and a peculiar deciduous one, which consists of *Salix Reinii*, *Alnus Maximowiczii*, *Acer Tschonoskii*, etc. associated with a vigorous undergrowth of *Sasa kurilensis*. It is interesting to note that this alpine deciduous shrub community is confined to the lee slope where snow lies late, whereas the first two are distributed on the other slopes (Figs. 1 and 2).

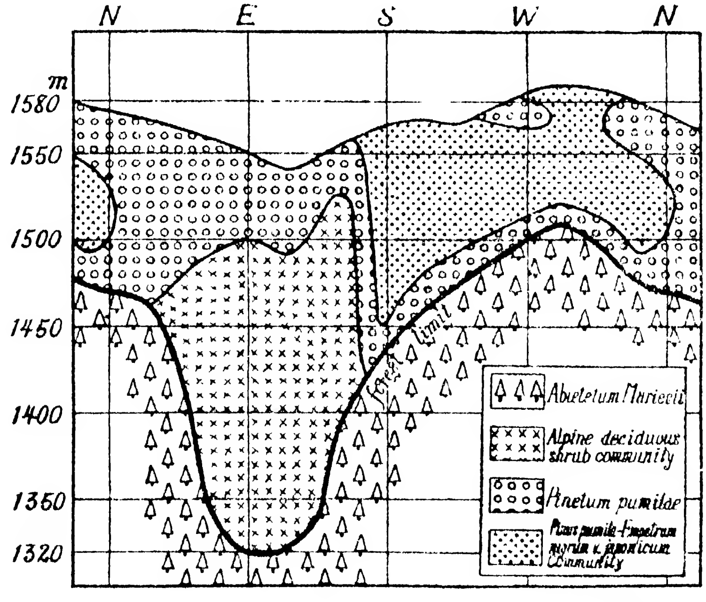

Fig. 2. Distribution of alpine shrub communities on Ôdake, in regard to the exposure and altitude.

Braun-Blanquet *et al.* (1932) has already dealt with the effects of snow lie on the distribution of ordinary alpine shrub and grassland communities in the Alps beside its effects on that of the characteristic snow-patch ones.

Yoshioka (1948) noticed also the community of tall growing herbs on the snow-rich leeward slope in this mountain, as Braun-Blanquet *et al.* did in the Alps. It is mainly composed of *Cacalia adenostyloides*, *Cacalia kamtschatica*, *Artemisia monophylla*, *Veratrum stamineum* var. *glabrum*, *Cirsium oligophyllum*, *Cirsium nipponicum*, *Veratrum japonicum* var. *Reymondianum*, etc.

Where snow remains so late that the aforementioned deciduous shrubs or tall growing herbs fail to develop, characteristic snow-patch communities do occur.

SOIL CONDITIONS OF SNOW-PATCHES

As has been stressed by Brockmann-Jerosch (1907), a vast quantity of water is supplied to the soil from the thawing snow-patch, and, when free from snow, the valleys and depressions where snow-patches lie receive undoubtedly abundant rain water. The excessively moist conditions of the soil together with the low soil temperature due to snow tends to prevent the decomposition of plant remains, and, as a result of this, soil becomes acid and peaty, as Lüdi (*see* Schröter 1926, p. 646) has already pointed out. Thus a gentle slope of defective drainage, in particular, is covered with a black peaty soil less than 20 cm. in depth (Soil-type 1). On the other hand, a steeper slope of good drainage is characterized by raw humus or loam rich in humus (Soil-type 2). At the very centre of a snow-patch, where snow remains until early autumn, plant remains do not accumulate and soil consists largely of fine sand which, according to Kerner (*see* Schröter 1926, p. 641), has presumably been air-borne to incorporate into snow (Soil-type 3). On a heap of debris at the foot of a cliff—"scree"—as is seen in the old crater of Ôdake, soil is brown loam admixed with gravels (Soil-type 4). The features of these four types of soil are shown in Table II.

Table II. Types of soil of snow-patches

Type	Topography	General feature	pH	Water content (%)	Loss on ignition (%)
I	Gentle slope of poor drainge	Black to brownish black, peaty	4.8	65.4	46.2
II	Steep slope of good drainage	Black, raw humus or loam rich in humus	4.7	54.7	31.9
III	The centre of snow-patches	Black, sandy	5.0	39.9	13.3
IV	Scree	Brown, loam admixed with gravels	5.2	40.6	14.6

As will be stated later, the type of soil of snow-patches is closely linked with the composition of the plant communities thereon supported.

FLORISTIC COMPOSITION OF SNOW-PATCH COMMUNITIES

Of the seventy-five species of plants found in the area of snow-patches, two mosses and a liverwort are confined to this habitat, namely, *Kiaeria falcata*, *Plagiothecium Roeseanum* and *Morckia nivalis*. Furthermore, there are several plants whose main habitat is the snow-patch soil, i.e. *Primula nipponica*, *Phyllodoce aleutica*, *Agrostis flaccida*, *Epilobium Dielsii*, *Sieversia pentapetala*, *Carex blepharicarpa*, *Tofieldia Okuboi*, *Arnica unalaschensis* var. *Tschonoskyi*, *Fauria Crista-galli*, *Peucedanum multivittatum* and *Gentiana nipponica*.

To compare the floristic composition of snow-patch communities with that of other formations on this mountain, Jaccard's coefficient of floral community (*Gemeinschaftskoeffizient*, *see* Jaccard 1932) is to be taken into account. As is seen in Table III, by far the highest coefficient is between these and the peat-bog formation, due no doubt to the high water content of both the soils. The alpine deciduous shrub community, too, which, as mentioned above, occupies the leeward slope rich in snow, shows a high value. Lower values are seen in two other alpine shrub communities flourishing on the windward slope where snow melts early. The forest floors of Fagetum crenatae, in particular, and Abietetum Mariesii, to a less extent, both composed chiefly of shade plants, have few species in common with the snow-patch communties.

Table III. The coefficient of floral community between snow-patch communities and other formations on Mt. Hakkôda

Other formations		Coefficient of floral community (%)	No. of common species/ No. of total species
Deciduous forest	Fagetum crenatae	6.2	10/161
Subalpine coniferous forest	Abietetum Mariesii	19.5	24/123
Alpine coniferous shrubbery	*Pinus pumila-Empetrum nigrum* var. *japonicum* community	23.0	20/ 87
The same	Pinetum pumilae	23.3	27/120
Alpine deciduous shrubbery		25.4	32/126
Peat bog		29.5	31/105

The life-forms after Raunkiaer of the snow-patch plants are listed in Table IV. It is there seen that most of the species belong to the hemicryptophytes.

Table IV. Life-forms of snow-patch plants

Life-form	Nanophanerophytes	Chamaephytes	Hemicryptophytes	Geophytes	Total
Numbers of species	8	7	43	13	75
Percentage	11	9	63	17	100

SOCIATIONS IN THE SNOW-PATCH COMMUNITIES

Thirteen sociations can be distinguished in the vegetation of snow-patches. One is dominated by a moss, one by a fern, two by dwarf shrubs and the other seven by grasses or herbs. In addition, two sociations dominated by shrubs are noticed on the border of snow-patches. The distribution of each sociation is closely bound up with the length of snow-free period—say the "growing period"—and the soil type.

(A) *Communities dominated by Mosses.* (1) *Kiaeria falcata* sociation:—This develops at the centre of snow-patches, where snow lies late and the growing period is as short as from 1/2 to 1 month, even none when snow remains until the next first snowfall.

The moss *Kiaeria falcata* is dominant in open places. But particularly in the crevices of rocks or in other moist shaded habitats, there also grow another moss *Plagiothecium Roeseanum* and a liverwort *Mörckia nivalis*. Among higher plants *Agrostis flaccida* (abundant) and *Epilobium Dielsii* (occasional) are characteristic of this sociation. *Primula nipponica* and *Fauria Crista-galli* are also found frequently. The soil is of type 3 and of pH 4.8 to 5.3. In spite of being situated in the course of thawing water, drainage is fairly good owing to its sandy texture.

That, as a rule, similar plant communities dominated by mosses or liverworts, namely, Polytrichetum sexangulare, P. norvegici, Antheletum, etc., occur at the centre of snow-patches was also brought out by BROCKMANN-JEROSCH (1907) and BRAUN-BLANQUET *et al.* (1932) in the Alps, by WATSON (1925) in the Scottish mountains, and by FRIES (1913) and GJAERFVOLL (1950) in the Scandinavian mountains.

(B) *Communities dominated by Grasses, Herbs or Dwarf Shrubs.* The vast majority of snow-patch communities fall in this category.

(1) ***Phyllodoce aleutica*** sociation:—This sociation dominated by the dwarf shrub occurs in the inner part of some snow-patches surrounding the *Kiaeria falcata* sociation mentioned above. *Tilingia ajanensis*, *Carex blepharicarpa*, *Peucedanum multivittatum*, *Gentiana nipponica* and *Tofieldia Okuboi* show high frequencies of more than 80 per cent in more than five quadrates of 2×2 m. in size. The growing period ranges from 1 to 2 months. The soil is of type 2 and of

pH 4.6 to 5.2. The surface soil is as thin as less than 10 cm., so that drainage is remarkable.

(2) *Sieversia pentapetala-Carex blepharicarpa* sociation:—This grows at the periphery of some snow-patches lying not so late, the growing period is from 2 to 3 months. The dwarf shrub *Sieversia pentapetala* and *Carex blepharicarpa* are co-dominant. Three typical snow-patch plants, i.e. *Peucedanum multivittatnm*, *Schizocodon soldanelloides* and *Gentiana nipponica*, occur with high frequency values of more than 80 per cent. Some plants whose chief habitat is outside the snow-patch such as *Pinus pumila* and *Diplycosia adenothrix*, mingle among them. The soil is of type 2 and of pH 4.6 to 4.7. Since the surface soil is less than 15 cm. in depth, drainage is relatively good.

(3) *Primula nipponica* sociation:—At the centre of some snow-patches with defective drainage, there thrives an open community of *Primula nipponica*. *Fauria Crista-galli* and *Tilingia ajanensis* have high frequency values of more than 80 per cent. Here the growing period is from 1 to 2 months. The soil is a peaty one of type 1 and the depth of surface soil measures from 15 to 20 cm., the pH falling within the range 4.6 to 4.9.

(4) *Carex blepharicarpa* sociation:—This, dominated by *Carex blepharicarpa*, flourishes close to the periphery of snow-patches with poor drainage, surrounding, as a rule, the above mentioned *Primula nipponica* sociation. Many snow-patch plants, such as *Primula nipponica*, *Fauria Crista-galli*, *Gentiana nipponica* and *Peucedanum multivittatum*, show high frequency values of about 80 per cent. The growing period ranges from 1.5 to 2.5 months. The peaty surface soil of type 1 is about 20 cm. deep and has a pH of 4.8.

(5) *Calamagrostis Langsdorfii-Carex blepharicarpa* sociation:—At the margin of some snow-patches, usually surrounding the above mentioned *Carex blepharicarpa* sociation, is noticed a community, whose composition is nearly the same as the former except for the co-dominance of *Calamagrostis Langsdorfii*. Plants usually found outside the snow-patch, let us say, *Pedicularis yezoensis* and *Hosta longissima* var. *brevifolia* develop prominently here. Next to this, outside the snow-patch, grow shrub communities. Here the growing period ranges from 2 to 3 months. The soil is of type 1, pH being 4.7 to 4.8.

(6) *Arnica unalaschensis* var. *Tschonoskyi* sociation:—This is found in the old crater of Ôdake on a dry scree fallen from the cliff, the growing period is 1.5 to 2 months. This is a pioneer community established particularly under the influence of snow-patches, and dominated by *Arnica unalaschensis* var. *Tschonoskyi*. The soil is of type 4 and of pH 4.9 to 5.6.

(7) *Calamagrostis Langsdorfii-Salix Reinii* sociation:—This surrounds the

aforementioned *Arnica unalaschensis* var. *Tschonoshyi* sociation. *Calamagrostis Langsdorfii* and *Salix Reinii* are co-dominant. This is a seral sociation (socies) succeeding the pioneer *Arnica unalschensis* var. *Tschonoskyi* sociation. Here the growing period is from 2 to 3 months, and the soil is of type 2 and of pH 4.6 to 4.9.

(8) *Schizocodon soldanelloides-Tilingia ajanensis* sociation.—This thrives in a depression on the north-east slope of Ôdake, where the growing period is from 2 to 3 months and the soil of type 2 with a pH of 4.6. Beside *Schizocodon soldanelloides* and *Tilingia ajanensis*, some other snow-patch plants, too, are abundant, showing a complex composition. Is is presumed to be a seral one.

(9) *Fauria Crista-galli-Carex geantha* sociation.—This distributes in the lower part of the subalpine zone (1000 m. in height), in contrast with the other sociations which are all situated in the middle or upper part of it or even in the alpine zone. The soil is of type 2 and of pH 4.5 to 5.0. *Fauria Crisia-galli* and *Carex geantha* are co-dominant. There also flourish *Lycopus parviflorus*, *Sanguisorba tenuifolia* var. *alba* and others, which are characteristic of peat bogs at this altitude.

(C) *Communities dominated by Ferns.* (1) *Thelypteris quelpaertensis* sociation.—This one, which is dominated by *Thelypteris quelpaertensis*, may be seen in the course of thawing water or at the periphery of snow-patches. The soil is of type 2 and of pH 4.9 to 5.0.

(D) *Communities dominated by Shrubs.* These are found on the very boundary of snow-patches and have different composition from those outside the snow-patch. The following two sociations may be distinguished, both co-dominated by *Botryostege bracteata*.

(1) *Vaccinium axillare-Botryostege bracteata* sociation.—This is supported by thin soil of type 2 and of pH 4.6 to 4.7. *Vaccinium axillare* and *Botryostege bracteata* are co-dominant.

(2) *Sasa kurilensis-Botryostege bracteata* sociation.—This one develops on thick and rather wet soil of type 2 and of pH 4.5 to 4.6. Dwarf *Sasa kurilensis* is co-dominant with *Botryostege bracteata*.

Of the foregoing thirteen sociations, the first six numbered A1 and B1 to B5 seem to be more or less stable, that is, to be regarded as subclimaxes, and, in reality, occupy the greater part of the snow-patch communities, whilst three others numbered B6, B7 and B8 are seral communities which are in process of succession having started from the bare land under the influence of snow-patches. D1 and D2 are shrub communities that lie on the boundary of snow-patches, forming a transitional zone to the communities outside of them.

In Table V are given the number of species which fall in the five frequency classes. It is there seen that most of the species in the six stable sociations belong to the class of the highest frequency. It follows from this that they are of homogeneous construction. On the contrary, the other sociations including the seral ones are considered to be of heterogeneous composition, since most of their species fall in the low frequency classes.

Table V. The number of species falling in five frequency classes in each sociation

Sociation	Frequency classes				
	V	IV	III	II	I
A1 : *Kiaeria falcata* sociation	4	2	0	0	2
B1 . *Phyllodoce aleutica* sociation	7	1	2	3	2
B2 : *Sieversia pentapetala-Carex blepharicarpa* sociation	12	2	3	4	3
B3 · *Primula nipponica* sociation	3	1	1	1	1
B4 : *Carex blepharicarpa* sociation	5	3	2	5	1
B5 : *Calamagrostis Langsdorfii-Carex blepharicarpa* sociation	7	2	3	2	1
B6 : *Arnica unalaschcensis* var. *Tschonoskyi* sociation	5	8	8	5	10
B7 : *Calamagrostis Langsdorfii-Salix Reinii* sociation	3	4	4	7	8
B8 : *Schizocodon soldanelloides-Tilingia ajanensis* sociation	6	5	5	2	6
B9 : *Fauria Crista-galli-Carex geantha* sociation	4	1	1	4	9
C1 : *Thelypteris quelpaertensis* sociation	2	1	1	5	8
D1 : *Vaccinium axillare-Botryostege bracteata* sociation	6	7	7	2	9
D2 : *Sasa kurilensis-Botryostege bracteata* sociation	6	3	3	5	8

ZONAL ARRANGEMENT OF THE SNOW-PATCH COMMUNITIES

Snow-patch communities show clear zonation from the innermost part to the periphery according to the length of the growing period, viz. the snow-free period, which is a fact observed also by many workers. The central part of a snow-patch, where snow melts latest, is occupied by plants which are most adapted to a short growing period, that is, by a community dominated by a moss (*Kiaeria falcata* sociation) or an open community of herbs (*Primula nipponica* sociation). On passing to the periphery, there occur closed herbaceous communities or those of dwarf shrubs, and finally a shrubbery on the border of the snow-patch. In Table VI are seen two representative types of zonation, which have developed on different types of soil.

The average number of species (specific density) counted in quadrates of 2×2m. in each sociation are given in Table VII. In every case except two shrub sociations where quadrates of 5×5m. were taken, this size of quadrates exceeds the minimal area. So the values as are seen in the table show the degree of complexity in the specific composition of these sociations. From comparison of Table VI with Table VII it will be obvious that a sociation situated at the centre of a snow-patch consists of few species and that the more remote the position of it the greater the number of species. That is to say, the longer the uncovered period the more pronounced the development of the community and the more complex is its floral composition.

Table VI. Two respresentative type of zonation in the snow-patches

<table>
<tr><th>Length of snow-free period</th><th>Nil</th><th colspan="4">to</th><th>3 months</th></tr>
<tr><td></td><td>Moss communities</td><td colspan="4">Herbaceous or dwarf-shrub communities</td><td>Shrub communities</td></tr>
<tr><td>Soil-types II and III</td><td><i>Kiaeria falcata</i> sociation (A1)</td><td colspan="2"><i>Phyllodoce aleutica</i> sociation (B1)</td><td colspan="2"><i>Sieversia pentapetala-Carex blepharicarpa</i> sociation (B2)</td><td><i>Vaccinium axillare-Botryostege bracteata</i> sociation (D1)</td></tr>
<tr><td>Soil-type I</td><td></td><td><i>Primula nipponica</i> sociation (B3)</td><td colspan="2"><i>Carex blepharicarpa</i> sociation (B4)</td><td><i>Calamagrostis Langsdorfii-Carex blepharicarpa</i> sociation (B5)</td><td><i>Sasa kurilensis-Botryostege bracteata</i> sociation (D2)</td></tr>
</table>

Table VII. Specific density of snow-patch sociations

Sociation	Specific density
A1 : *Kiaeria falcata* sociation	6.5
B1 : *Phyllodoce aleutica* sociation	13.0
B2 : *Sieversia pentapetala-Carex blepharicarpa* sociation	15.6
B3 : *Primula nipponica* sociation	6.2
B4 : *Carex blepharicarpa* sociation	11.5
B5 : *Calamagrostis Langsdorfii-Carea blepharicarpa* sociation	14.5
B6 : *Arnica unalaschensis* var. *Tschonoskyi* sociation	16.6
B7 : *Calamagrostis Langsdorfii-Salix Reinii* sociation	15.8
B8 : *Schizocodon soldanelloides-Tilingia ajanensis* socintin	15.8
B9 : *Fauria Crista-galli-Carex geantha* sociation	9.0
C1 : *Thelypteris quelpaertensis* sociation	9.6
D1 : *Vaccinium axillare-Botryostege bracteata* sociation	16.1
D2 : *Sasa kurilensis-Botryostege bracteata* sociation	15.4

SUMMARY

1. Plant communities developed after snow-patches have melted—"snow-patch communities"—are, for the most part, to be regarded as physiographic subclimaxes caused by a local abundance of snow accumulation which is closely

related to the physiography.

2. On Mt. Hakkôda, snow-patches are confined to leeward slope facing the east or south-east, owing to the prevailing west or north-west winds in winter. This is considered to account for that the forest limit is some 200 m. lower on the leeward slope than on the windward, and for that the alpine shrub community above the forest limit are deciduous on the leeward slope in contrast with the coniferous ones on the other slopes.

3. Characteristic snow-patch communities are found where the snow-free period is less than three months, so that even the deciduous shrubs mentioned above fail to develop. Sometimes snow remains till early autumn at the centre of a snow-patch, and, in years of heavy snowfall, even till the first snowfall to come. As to the altitude, they are distributed only on slopes higher than the lower part of the subalpine zone, that is, from 1000 m. above sea-level onwards.

4. Beside the restriction in the growing period, abundance of water supply to the soil is an outstanding feature of a snow-patch.

5. Soil of snow-patches falls into four types which are markedly related to the distribution of a variety of communities.

6. Many of snow-patch plants are considered to flourish chiefly in the snow-patch, whilst two mosses and a liverwort are altogether confined to it.

7. There are a number of species in snow-patch communities which are common with the alpine deciduous shrub community on the snow-rich slope and the peat-bog formation. Preponderance of hemicryptophytes is to be noted.

8. In the snow-patch communities are distinguished thirteen sociations, of which six are presumably stable, that is to say, to be regarded as subclimaxes, and other three seral. One is dominated by a moss, one by a fern, two by dwarf shrubs and the other seven comprising the seral ones are predominated by grasses or herbs. Moreover, two shrub sociations bound the snow-patch.

9. A clear zonation is noticed in the snow-patch communities. The innermost part is occupied by a moss community and the outer by the herbaceous, dwarf shrub and shrub communities. The floristic composition of the communities become simpler as passing to the centre.

In conclusion, the author wishes to express his hearty thanks to Prof. Dr. Y. YOSHII, under whose direction this study was carried out.

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STUDIEN ÜBER DIE BILDUNG ORGANISCHER SÄUREN IN GRÜNEN PFLANZEN
III. ÜBER DAS VERHÄLTNIS ZWISCHEN DEM SÄUREGEHALT UND DEM N- UND C-UMSATZ VON *BEGONIA EVANSIANA* ANDR. (FORTSETZUNG)

VON

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(Mit 6 Textfiguren)

VII. ÜBER DEN EINFLUSS DES KOHLENHYDRATMANGELS AUF DIE SÄUREBILDUNG IN DEN BLÄTTERN

In der ersten Mitteilung dieser Serienpublikation versucht ich unter Durchleitung der CO_2-freier Luft zu erkennen, ob und wie die Assimilation der Kohlensäure die Säurebildung beeinflusst. Das wichtigste Ergebnis damaliger Untersuchungen bestand wohl darin, dass bei den in kohlensäurefreier Luft gezogenen Pflanzen der Säuregehalt der Blattspreite und des Blattstiels vom unteren Knoten nach dem oberen hin abnahm. Bei der Kontrollpflanze verlief aber die Reihenfolge des Säuregehalts in den genannten Organen in ganz umgekehrter Richtung gegen den obigen Fall, während sie sich in den Stengel einschliesslich der betreffenden Knoten in der gleichen Richtung wie bei den in CO_2-freier Luft gezogenen fand, was sich einen Gegensatz zur damals erkannten allgemeinen Reihenfolge darstellen sollte. Also liegte es noch sehr durstig nach die Feststellung, ob sich diese Erscheinung immer dort finden sollte, wo eine Hemmung der Kohlensäureassimilation herbeigeführt würde.

Versuch 26. Kontrollpflanze. (1933)

Dieser Versuch dient als Kontroll zu den nachfolgenden Versuchen 27 und 28. Die Versuchspflanze (Nr. 42) wurde 180 Tage im Gewächshaus und dann 12 Tage lang unter der Glocke unter Durchleitung der gewöhnlichen Luft künstlich gezogen. Nach diesen Kulturdauer wurde das Material abgenommen und in einem und demselben Pflanzenteile sofort der Säure- und N-Gehalt bestimmt. Zur Bestimmung der Säure- bzw. N-Menge wurden die Abschnitte von 0.078–0.342g bzw. 0.211–1.168g genommen. Der Versuchsvorrichtung und der Analysengang sind im folgenden immer dieselben geblieben, wie in den ersten (1932) und zweiten (1933) Mitteilungen dieser Serie angegeben sind. Die einzelnen Befunde sind in Fig. 6

und die durchschnittlichen Werte in Tabelle XXVII zusammengestellt. Wie man sieht, ist der Säuregehalt im Blattstiel grösser als in der Blattspreite und nimmt von der unteren Knoten nach oberen hin stark zu (Fig. 6), wie wir vorher erfahren haben. Beim Stengel einschliesslich der betreffenden Knoten nimmt er dagegen gerade in umgekehrter Richtung von der Basis nach der Spitze hin ab. Dieser Tatsache stimmt mit der früheren Befunde gut überein (s. SHIBATA 1932). Diese Reihenfolge der Säuremenge entlang dem Stengel bzw. den betreffenden Blattspreiten und -stielen war damals unerwart, aber haben wir dabei gedeutet, dass diese Erscheinung auf der Rückwanderung der Säure in die Knollen oder auf entgegengesetzten Stoffwechselvorgänge in diesen Pflanzen beruhen können, sodass sie im allgemeinen gerade da hatten vorkommen sollen, wo nun das Wachstum zum Stillstand komme. Das Vergleich dieser und einer später zu erwähnenden Fällen, wo die Pflanze sehr lang über 170 Tage in Kultur gewesen sind, mit den Fällen der jüngeren Pflanzen, wie man in meiner früheren Untersuchung zu finden sind (SHIBATA 1932, 1933), zeigt es klar, dass der obengenannten Deutung die Richtigkeit zugeschrieben werden muss. Zwar handelt es nur um die Entwicklungsphase, ob man eine oder die andere Reihenfolge der Säuremenge entlang dem Stengel gefunden hätte.

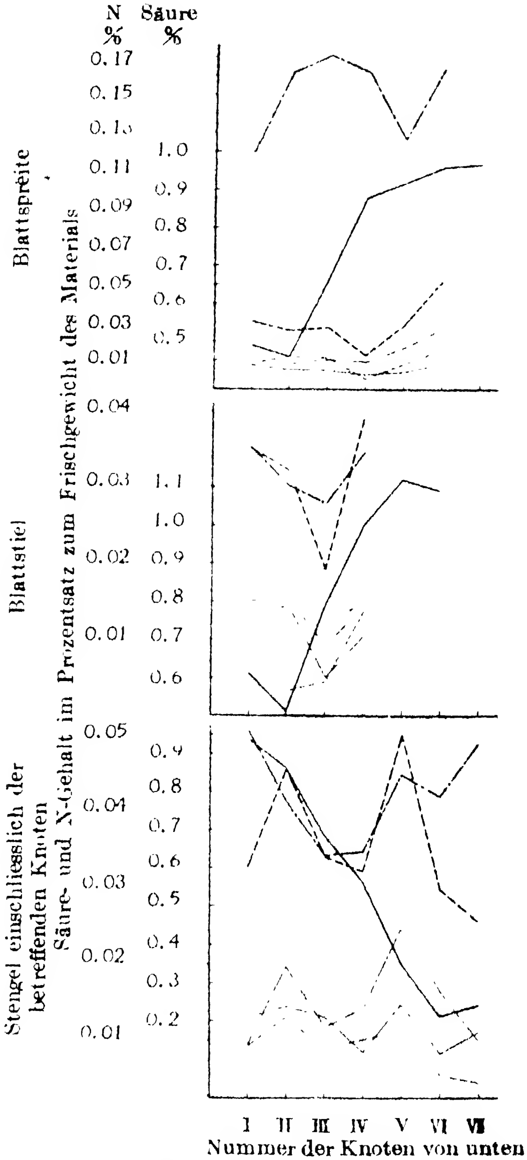

Fig. 6 Säure- und N-Gehalt von *Begonia Evansiana* ANDR. (Kontrollversuch).

———— Säure ———— Ammon-N
—·—·— Eiweiss-N —··—··— Amid-N
- - - - lösl.-N -·-·- Amino-N

Was den Gehalt des Gesamt-N bezogen auf 1000g Frischgewicht des materials

Tabelle XXVII. Der Einfluss des Kohlenhydratmangels auf den Säure- und N-Stoffwechsel—Kontrollversuch

Pflanzenteil		Blattspreite	Blattstiel	Stengel	in ganzen Pflanzenkörper
mg Oxalsäure in 1000g Frischgew.		7634.5	8548.6	5481.4	7221.5
mg N in 1000g Frischgew.	Gesamt-N	1819.2	636.5	779.0	1078.2
	Eiweiss-N	1518.5	322.4	421.1	754.0
	lösl.-N	300.7	314.1	357.9	324.2
	Ammon-N	55.8	104.9	117.3	92.7
	Amid-N	147.6	135.4	150.8	144.6
	Amino-N	97.3	73.8	89.8	86.9
Amid/Ammon		2.71	1.43	1.32	1.82

anbetrifft, so ist er bei Blattspreite 2 mal gross wie sowohl beim Stengel einschliesslich der betreffenden Knoten als auch beim Blattstiel (s. Tabelle XXVII). Ähnlich verhält sich der Eiweiss-N, indem dessen Gehalt bei der Blattspreite 3–5 mal so gross wie der bei den anderen Teilen. Inbetreff des löslichen N ist das Verhältnis umgekehrt. In bezug auf den absoluten Gehalt an einzelnen löslichen N-Fraktionen in 1000g Frischgewicht ist die Menge des Ammon-N bei dem Blattstiel und dem Stengel einschliesslich der betreffenden Knoten doppelt so gross als die bei der Blattspreite. Der Amid-N bildet der Hauptbestandteile (40–50%) des löslichen N und weist er zwischen den verschiedenen Organen wenig Unterschied auf. Der Gehalt am Amino-N zeigt bei den verschiedenen Organen wenig Unterschied. Der Quotient Amid/Ammon (0.75–3.85) entspricht dem der Säurepflanze, und ist er bei Blattspreite grösser als beim Blattstiel und Stengel einschliesslich der betreffenden Knoten.

Versuch 27. Pflanze in CO_2-freier Luft gezogen. (1933)

Das eine Individuum (Nr. 24) wurde nach der 170 tägigen Züchtung im Gewächshaus 12 Tage lang in kohlensäurefreier Luft gezogen. Die 1. und 6. Blattsielen waren aber nicht gross genug, um daran die Bestimmung sowohl des Säure- als auch des N-Gehalts nebeneinander durchgeführt zu werden, sodass bei ihnen entweder der einen oder der anderen bestimmt wurde. Zur Bestimmung der Säure- bzw. N-Menge wurden die Abschnitte von 0.032–0.356g bzw. 0.232–1.203g verwendet.

Die einzelnen Versuchsergebnisse sind Fig. 7 und die durchschnittlichen Zu- oder Abnahmen betreffender Werte in Tablle XXVIII zusammengestellt. Hier ist aber zu bemerken, dass die beiden Versuchspflanzen (Nr. 4 und 42 (Kontrolle)) trotz der ähnlichen Entwicklungsstadien doch nicht in derselben Stoffwechselvorgängen befinden können, sodass die hier gegebenen Vergleichsdarstellung der Zu- oder

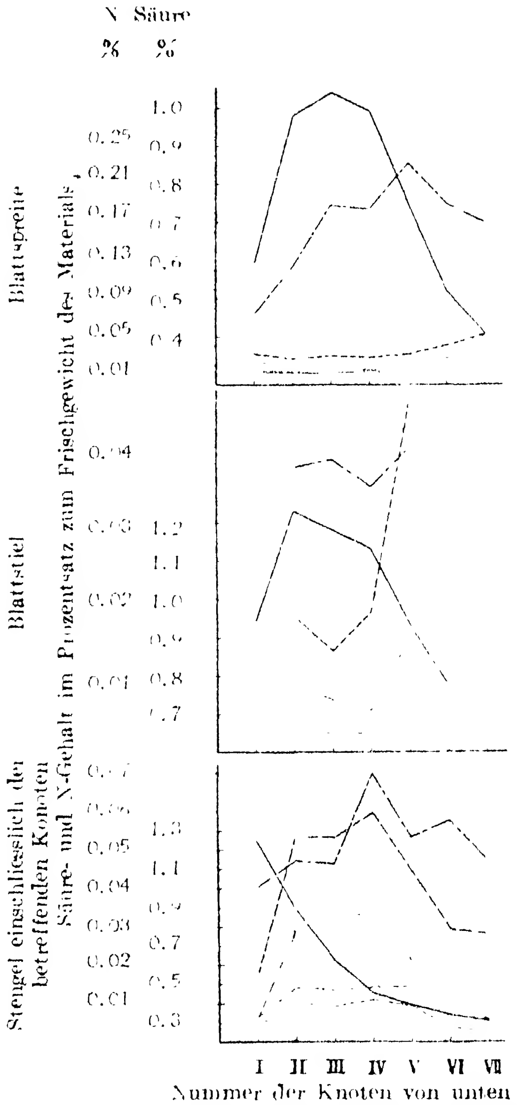

Fig. 7. Säure- und N-Gehalt von *Begonia Evansiana* Andr. (CO_2-frei-Versuch).

——— Säure ——— Ammon-N
—·— Eiweiss-N -·-·— Amid-N
— — — lösl.-N -·-·-·- Amino-N

Abnahme der betreffenden Werte streng genommen nicht auszuführen ist. Aber die blosse Analyse der gefundenen Werte würde nicht durchsichtig erscheinen, da wir augenblicklich mit der Zu- oder Abnahme der Säure bzw. der verschiedenen N-Werte unter den CO_2-freien Bedingungen beschäftigen. Aus Tabelle XXVIII ersieht man, dass der durchschnittlichen Säuregehalt des Blattstiels wie immer grösser als bei den anderen Organen ist, während er in den Blattspreiten sogar zum einen Verlust unterworfen ist. An Fig. 7 kann man weiter sehen, dass die Säuremenge in ganz jungen Blattspreiten und -stielen, entgegen der Kontrollpflanze, stark abgenommen sind. In jedem Falle its aber bei drei betreffenden Pflanzenteilen die allgemeine Neigung zur Abnahme des Säuregehalts der Reihenfolge nach der Knoten von der Basis nach der Spitze zu wahrzunehmen. Diese Erscheinung stimmt mit früheren Resultate (Shibata 1932) gut überein, und daran die Züchtung in CO_2-freier Luft schuldig sein muss. Dass der Säuregehalt der Blattspreite und des Blattstiels in ihrer ältesten Entwicklungsstadien sehr gering war, stellt einen Gegensatz zum Stengel dar, wozu sehr wahrscheinlich das Gelbwerden der Blätter verantwortlich sein kann. Was den Gehalt an Gesamt-N bezogen auf 1000g Frischgewicht des Materials anbetrifft, so ist er im allgemeinen bei den Stengeln am grössten und bei Blattspreiten sogar von einen kleinen Verlust erlitten. Der Eiweiss-N neigt, wie aus Fig. 7 zu entnehmen ist, Tabelle XXVIII. Der Einfluss des Kohlenhydratmangels auf den Säure- und N-Stoffwechsel—CO_2-frei-Versuch

Pflanzenteil		Blattspreite	Blattstiel	Stengel	in ganzen Pflanzenkörper
mg Oxalsäure in 100g Frischgew.		−61.2	+1760.9	+505.5	+761.7
mg N in 100g Frischgew.	Gesamt-N	−9.1	−20.1	+143.6	+38.1
	Eiweiss-N	+44.9	+52.3	+94.5	+63.9
	lösl.-N	−54.0	−72.4	+49.1	−25.8
	Ammon-N	−29.4	−58.6	−45.9	−44.0
	Amid-N	+5.2	−17.8	+74.0	+20.4
	Amino-N	−29.8	+4.0	+21.0	−1.6
Amid/Ammon		6.86	2.83	3.76	

von der Basis nach der Spitze hin zuzunehmen. Diese Neigung kann man auch beim löslichen N wahrnehmen. Absolut genommen sind aber die Schwankungen bei einzelnen Fraktionen der löslichen N so klein, dass sie in ihrer Bedeutung zur Säureschwankungen ganz und gar vernachlässigt werden könnten. Wenn also, die Säurebildung in irgendeiner Beziehung zum N-Stoffwechsel stehen sollte, bleibt nur der Eiweiss-Stoffwechsel in Betracht gezogen zu werden. Wie man aus Tabelle XXVIII entnehmen kann, nahm der Gesamt-N während der CO_2-freien Züchtung dem Kontrolle entgegen mässig zu, darunter der Eiweiss-N-Zunahme Hauptrolle spielt. Diese Tatsache, mit der durchgehenden Ammon-N-Abnahme zusammen, deutet dahin, dass hier die Eiweissynthese stattfindet, ganz wie bei meiner früheren Orientierungsversuche der Fall war (Shibata 1932). Wie man auch unten zu erfahren ist, die Kohlenhydtate der Pflanzen unter CO_2-freier Luft, entgegen die bei den unter Dunkel gezogenen Pflanzen, erleiden wenig an ihrem Mangel; sie können ja sogar ganz unverändert bleiben, je nach dem Dauer der Züchtung unter der genannten Bedindung. So ist es ganz begreiflich, dass bei der Pflanze, die die N-Verbindungen aus Nährlösung aufnehmen kann, Eiweissynthese unverzögert stattfinden kann. Weil die Mengenverhältnis der löslichen N-Fraktionen zur Säure nicht für Desaminierungshypothese spricht, muss man aus dieser Ergebnisse etwa so vorstellen, dass der Mechanismus der Säurebildung, wenn sie zum Teil mit dem N-Stoffwechsel in kausaler Beziehung stehen sollte, vielmehr in dem mit der Eiweissynthese geköppelten Stoffwechsel zu suchen wäre. Der Quotient Amid/Ammon ist etwas zum Amid verschoben und beim Blattstiel am kleinsten, beim Stengel einschliesslich der betreffenden Knoten ein mittlerer und bei der Blattspreite am grössten. Es ist ersichtlich, dass zwischen dieser Verhältniszahl und den absoluten Gehalt der gesamten Säure eines umgekehrtes

Verhältnis besteht, wie KULTZSCHER (1932) zwischen Amid/Ammon und pH dasselbe Verhältnis feststellen konnte.

Versuch 28. Pflanze im Dunkeln gezogen (1933)

In den ersten Mitteilung habe ich über den Säuregehalt der im Dunkel gezogenen etiolierten Pflanze, die gleich nach der Keimung von Bulbillen im Keller weiter kultiviert wurde, eine Kundschaft gemacht.* (SHIBATA 1932) In diesem Falle nahm der prozentuale Säuregehalt im Stengel einschliesslich der Knoten, im Blattstiel und in der Blattspreite ausnahmslos von der Basis nach der Spitze hin zu. Zum selben Zweck wie beim vorigen wurde weiter ein anderes Individuum (Nr. 9) analysiert. Es war 173 Tage im Gewächshaus und dann 12 Tage lang unter ca. 20L-haltiger Glocke, die zuerst mit schwarzer Emailfarbe, um die Versuchspflanze vor Lichtzutritt zu schützen, und dann zur Verminderung der Absorption der Sonnenstrahlenwärme mit weisser bestrichen war, unter Durchleitung der Luft gezuchtet.

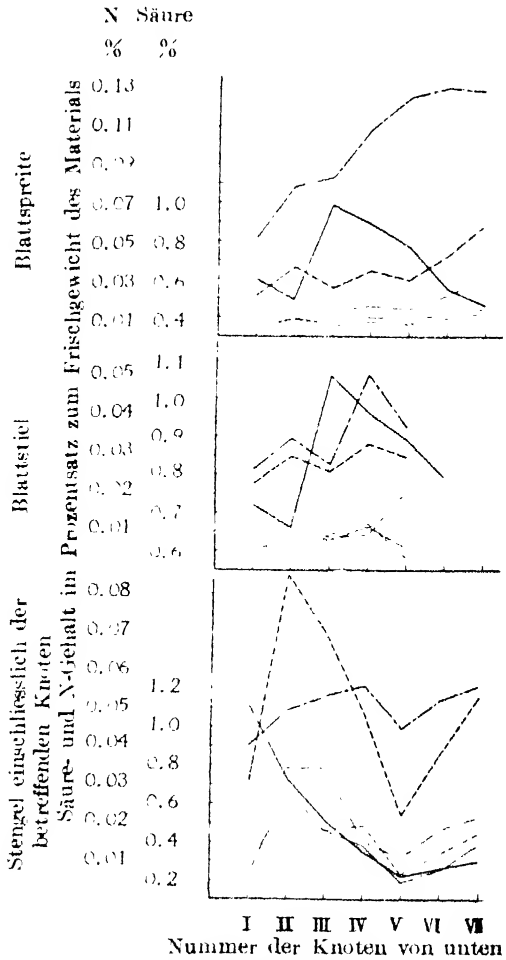

Fig. 8 Säure- und N-Gehalt von *Begonia Evansiana* ANDR. (Verdunklungsversuch).

——— Säure ——— Ammon-N
— - — Eiweiss-N — - — Amid-N
- - - - lösl.-N - - — Amino-N

Zur Bestimmung der Säure- bzw. N-Menge wurden die Abschnitte von 0.073–0.338 g bzw. 0.185–1.012 g verwendet. Die einzelnen Analysenergebnisse sind in Fig. 8 graphisch angegeben, und die durchschnittlichen Daten sind in Tabelle XXIX zusammengestellt. Aus dieser Figur ersieht man, dass der absolute Säuremenge des Blattstiels immer grösser als der der Blattspreite ist, und dass er im Stengel einschliesslich der betreffenden Knoten mittelständig ist, ganz wie wir beim Versuch 25 erfahren haben. Aus Fig. 8 kann man ferner sehen, dass sich die Säure in ganz jungen Blattspreiten und Blattstielen geringer als in mässig gewachsenen findet. In jedem Falle ist die Säuremenge sowohl bei der

Tabelle XXIX. Der Einfluss des Kohlenhydratmangels auf den Säure- und N-Stoffwechsel—Verdunklungsversuch

Pflanzenteil		Blattspreite	Blattstiel	Stengel	in genzen Pflanzenkörper
mg Oxalsäure in 1000g Frischgew.		—817.7	—63.4	—504.5	—461.8
mg N in 1000g Frischgew.	Gesamt-N	—449.0	+1.4	+219.3	—76.1
	Eiweiss-N	—503.4	+33.3	+81.3	—129.6
	lösl.-N	+54.4	—31.9	+138.0	+53.5
	Ammon-N	+23.1	—26.9	+14.8	+3.6
	Amid-N	+36.5	—2.6	+61.6	+31.8
	Amino-N	—5.2	—2.4	+61.7	+18.1
Amid/Ammon		2.52	1.62	1.84	

Blattspreite als auch beim Blattstiel an mitteren Körperteilen grösser als der beim oberen oder unteren. Der geringere Säuregehalt des Blattstiels und der Blattspreite bei den 1. und 2. Knoten beruht offenbar auf das Gelbwerden des Blattes und kann der Zersetzung oder Ablenkung der Säure an anderen Körperteilen der Versuchspflanzen zurückgeführt werden. Im Stengel erlitt die Säure, ganz wie bei der Kontrolle, von Basis zur Spitze von einer allmähligen Abnahme. Im Grossen und Ganzen kann man aber gleich bemerken, dass die Säure bei jüngeren Blattspreiten und -stielen stark abnahm, was ja ganz und gar mit den Ergebnissen bei der CO_2-freien Züchtung übereinstimmt, und einen deutlichen Gegensatz zum Kontrolle darstellt. Der Quotient Amid/Ammon beträgt 0.83–2.66 ähnlich wie bei der Kontrolle, und beim Blattstiel ist er am kleinsten, beim Stengel einschliesslich der betreffenden Knoten mittelständig und bei Blattspreite am grössten. Aus diesem Ergebnis ist also gleich erkennbar, dass die Säurebildung durch die längere Verdunklung überall gestört wird. Dagegen die jeden einzelnen löslichen N-Fraktionen sind beim ganzen Körper der Dunkelpflanzen grösser als die bei der Kontrolle (s. Tabelle XXIX), was mit dem niedrigeren Wert Eiweiss-N zusammen auf den Abbau des Eiweisskörpers hindeutet. Trotz dieses höheren Gehalts an löslichen N bei den Dunkelpflanzen nimmt die Säuremenge umgekehrt ab. Beachtenswert ist weiter die Tatsache, dass sich die Kohlenhydrate, u. a. der Zucker und die Stärke, wie man im späteren Abschnitt erfahren wird, im 12tägigen Verdunklungsversuch grösstenteils verschwinden, was trotz der äusseren Ähnlichkeit ein wichtiger Gegensatz zum CO_2-freien Versuch darstellt. Also steht die Sache so, dass beim CO_2-freien Versuche die Kohlenhydratmenge fast unverändert bleibt, und die Säure- und Eiweissbildung etwa gefördert werden können, während sie sich beim Verdunklungsversuche grösstenteils verschwinden, sodass die Säure- und Eiweissbildung wenn überhaupt beeinträchtigt werden müssen. Da wir als Tatsache die Säureabnahme gefunden haben, was sicher wegen des Kohlenhydratmangels die Beteiligung der Säure an Energielieferung hindeutet, es ist schwer zu entscheiden, ob man hier die Säurebildung in Rahmen der Desaminierungshypothese einstellen könnte.

Versuch 29. Kontrollpflanze. (1934)

Zum selben Zweck, wie Versuch 26, wurde weiter ein anderes Individuum

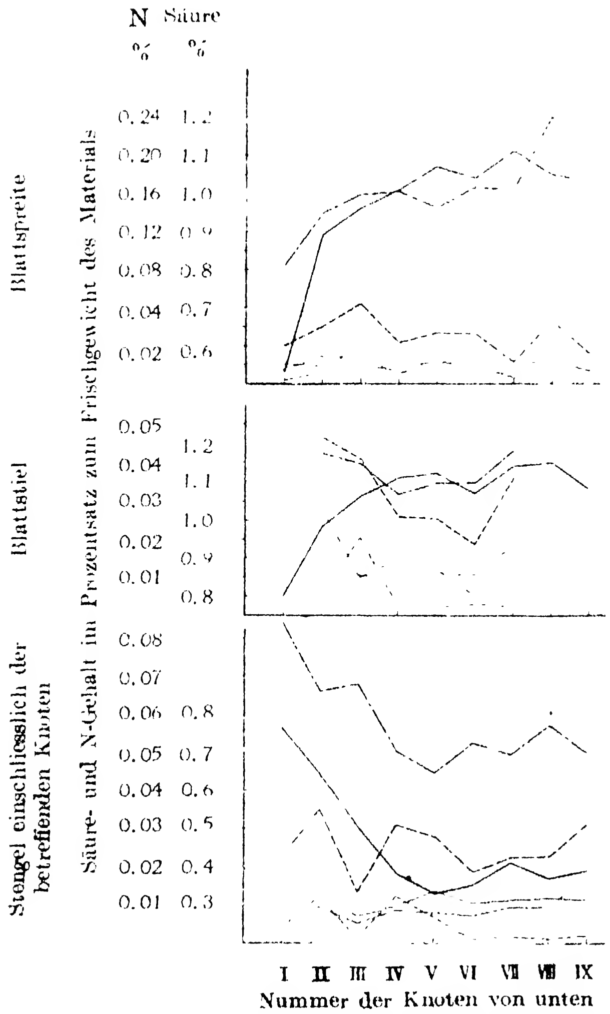

Fig. 9. Säure- und N-Gehalt von *Begonia Evansiana* ANDR. (Kontrollversuch).

Säure
Eiweiss-N
lösl.-N
Ammon-N
Amid-N
Amino-N

(Nr. 18) für 174 Tage lang im Gewächshaus, dann für 21 Tage lang unter Glocek unter Durchleitung der Luft künstlich gezüchtet, was schon ausgeblüht war. Das 1. Blatt war schon blassgrün und 2 und 3. Blatt gelblichgrün geworden, aber übrige Blätter waren schwarzgrün und anscheinlich noch ganz gesund. Zur Analyse wurden die Abschnitte von 0.076–0.460g für Säure und die von 0.349–1.604g für N genommen. Die Ergebnisse sind in Fig. 9 und durchschnittliche Werte in Tabelle XXX wiedergegeben. Daran kann man leicht erkennen, dass die Schwankungen aller Daten vollkommen der der Fig. 6 übereinstimmen, sodass die eingehende Auseinandersetzung überflussig wäre.

Tabelle XXX. Der Einfluss des Kohlenhydratmangels auf den Säure und N-Stoffwechsel—Kontrollversuch (1934)

Pflanzenteil		Blattspreite	Blattstiel	Stengel	in ganzen Pflanzenkörper
mg Oxalsäure in 1000g Frischgew.		9961.8	10582.5	4599.5	8283.5
mg N in 1000g Frischgew.	Gesamt-N	1882.7	699.3	825.7	1135.9
	Eiweiss-N	1628.5	377.4	577.4	861.0
	lösl.-N	254.2	321.9	248.6	274.9
	Ammon-N	99.8	108.6	106.6	102.0
	Amid-N	108.3	154.5	64.9	119.2
	Amino-N	56.1	53.8	47.1	52.6
Amid/Ammon		1.35	1.47	0.90	1.24

Versuch 30. Pflanze in CO_2-freier Luft gezogen (1934).

Um die 1933 erhaltenen Ergebnisse zu kontrollieren, wurde weiter analysiert ein Individuum Nr. 18, das für 145 Tage lang im Gewächshaus, dann für 14 Tage

Tabelle XXXI. Der Einfluss des Kohlenhydratmangels auf den Säure- und N-Stoffwechsel—CO_2-frei-Versuch (1934)

Pflanzenteil		Blattspreite	Blattstiel	Stengel	in ganzen Pflanzenkörper
mg Oxalsäure in 1000g Frischgew.		−3067.3	−2686.6	+824.0	−1643.3
mg N in 1000g Frischgew.	Gesamt-N	−23.1	+148.8	+20.7	+48.8
	Eiweiss-N	−266.1	−6.2	−171.2	−147.8
	lösl.-N	+243.0	+155.0	+191.9	+196.6
	Ammon-N	−198.7	+141.1	+161.2	+167.0
	Amid-N	+19.5	−1.8	+40.0	+20.4
	Amino-N	+24.8	+12.1	−9.3	+9.2
Amid/Ammon		0.67	0.71	0.57	

lang unter Glocke unter Durchleitung der CO_2-freier Luft künstlich gezogen wurde, und schon ausgeblüht war. Das 1. Blatt wurde schon gelb, andererseits die 7. und 8. Blättern waren nicht gross genug, um die Bestimmung sowohl des Säure- als auch des N-Gehalts nebeneinander durchzuführen, sodass bei ihnen immer nur der Säuregehalt bestimmt wurde. Zur Bestimmung der Menge von Säure bzw. N wurden die Abschnitte von 0.093–0.470 bzw. 0.333–1.535g verwendet. Die einzelnen Analysenergebnisse sind in Fig. 10 und die durchschnittlichen Werte in Tabelle XXXI angegeben.

An der Fig. 10 ist ersichtlich, dass die Kurven der Säureschwankungen (an Blattspreite und -stiel) ganz gut mit der der 1933jährigen übereinstimmen. Die Kurven der Gesamt-N und andere N-Fraktionen laufen ebenso ähnlich wie die der 1933 jährigen. Bezüglich des absoluten N-Gehalts bezogen auf 1000g Frischgewicht des Materials ist der Gesamt-N bei der Blattspreite am grössten und betrug etwa doppelt so gross wie der bei anderen Organen. Bei der Blattspreite macht der Eiweiss-N sogar zwei Drittel des Gesamt-N aus, während er bei anderen Organen beinah der Hälfte des Gesamt-N reicht. Der absolute Gehalt an löslichen N bei verschiedenen Organen verhält sich

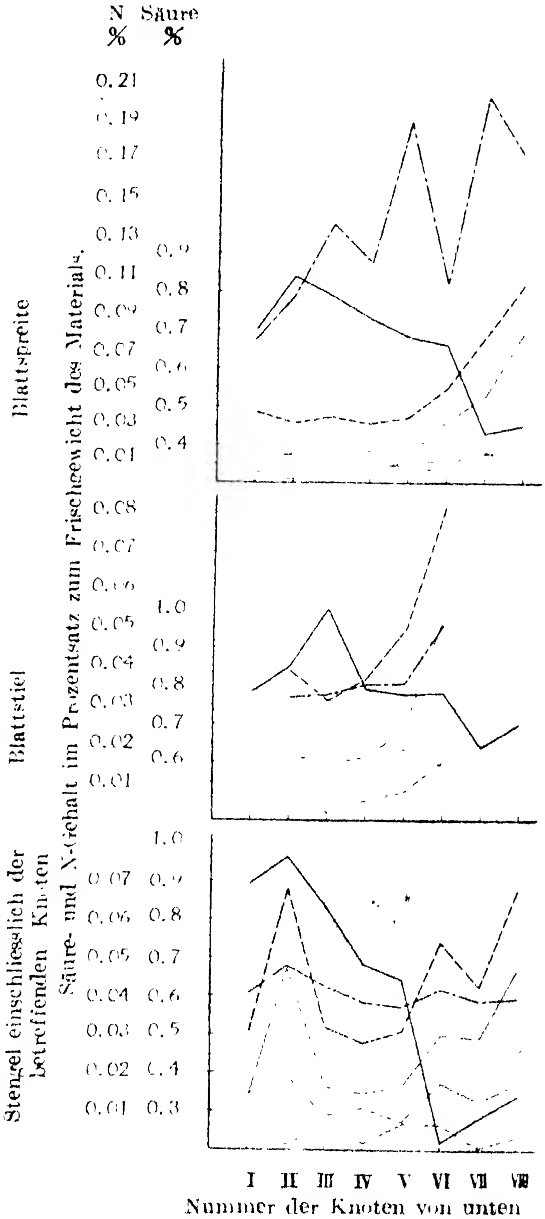

Fig. 10 Säure- und N-Gehalt von *Begonia Evansiana* ANDR. (CO_2-frei-Versuch).

——— Säure ——— Ammon-N
— - — Eiweiss-N — - — Amid-N
– – – lösl.-N – – – Amino-N

ganz umgekehrt. Unter dem absoluten Gehalt an einzelnen Fraktionen des löslichen N in jeden betreffenden Pflanzenteilen nimmt der Ammon- und Amino-N im allgemeinen von der Basis zur Spitze hin zu, was ganz umgekehrtes Verhältnis zur Schwankung der Säuremenge der Pflanzenachse entlang darstellt. Überdies hier findet sich der Ammon-N beinah doppelt so reich wie der bei der Kontrolle, während bei anderen löslichen N-Fraktionen der Unterschied zwischen beiden kaum zu erkennen ist. Alle diese deuten klar dahin, dass die Eiweisspaltung bzw. Desaminierung ansehnlich stattgefunden ist. Der Quotient Amid/Ammon schwankt 0.19–1.74, sodass er viel kleiner als bei der Kontrollpflanze ist. Wenn man die absoluten Säuremenge der jeden einzelnen Pflanzenteilen auf dem Ordinat und die entsprechenden absoluten Menge Eiweiss- oder löslichen N in 1000g Frischgewicht auf dem Abzisse entgegen in einer Kurve darstellt, kann man leicht ersehen, dass die Kurven für Blattstiel und Stengel bei diesem Versuch beinah gleich Richtung wie die entsprechenden Kurven beim Kontrollversuch richten. Bei der Blattspreite ist es auch hier augenfällig, dass die Säuremenge mit den zunehmenden Menge Eiweiss-N umgekehrt abnimmt. Der Gesamt-N-Gehalt beträgt bei beiden Versuchspflanzen durchschnittlich 0.19% des Frischgewichtes. Da nun der prozentuale Gehalt an Eiweiss-N gegen den Gesamt-N 73.56% bei diesen Versuchspflanzen gegenüber 86% bei der Kontrolle, und 26.55%iger Gehalt an löslichen N gegen 13.99% bei der Kontrollpflanze einnimmt, es deutet augenscheinlich dahin, dass bei jener die Eiweisspaltung kräftiger eingetreten ist. Dass die Säure bei dieser Versuchspflanze trotz des grösseren Eiweissabbau weniger gefunden wurde, kann ohne weiteres nach der Theorie Ruhland's nicht erklärt werden. Damit kann man trotzdem noch im Stande sein, die genannte Theorie einfach und eindeutig zu verneinen, weil sich hier die Versuchspflanze unter anderer inneren Bedingungen als die Kontrollpflanze befunden sein und die anscheinliche Säureabnahme in der Wirklichkeit die Zunahme gewesen sein könnte. Etwa so, dass die schon beim Versuch die Kohlenhydrate verhältnismässig weniger als die Kontrollpflanze enthielt, sodass die Säure selbst inzwischen als Atmungsmaterial verbraucht werden müsste. Aus diesem Grunde bleibt darüber noch weiterer Untersuchungen durstig.

Versuch 31. Pflanze im Dunkel gezogen. (1934).

Zum demselben Zweck, wie in 1933 ausgeführten Versuchen 28, wurde ein Individuum Nr. 25 analysiert, das 196 Tage lang im Gewächshaus, dann 14 Tage lang unter Glocke, die vor Lichtzutritt schützt, unter Durchleitung der Luft künstlich bis zum Ausblühen gezogen wurde. Allen Blattspreiten waren grün und anscheinlich gesund. Zur Bestimmung der Menge von Säure bzw. N wurden die Abschnitte von 0.095–0.417 bzw. 0.210–1.288g verwendet. Die einzelnen und durchschnittlichen Analysenergebnisse der Säure- und N-Bestimmung sind je in Fig. 11 und Tabelle XXXII wiedergegeben. Fig. 11 zeigt, dass der Säuregehalt beim Blattstiel wie immer am grössten und beim Stengel einschliesslich der betreffenden Knoten am kleinsten ist. Überdies ist an der Fig. 11 ersichtlich, dass der Säuregehalt bei allen Pflanzenteile nach dem Knotennummer entlang von der Basis nach die Spitze hin abnimmt, was zu den Ergebnissen bei der Kontrollpflanze soweit entgegen steht, als sie bei dieser wenigstens in den älteren Blattspreiten und -stielen umgekehrt zuhahm. Was den absoluten Gehalt an Gesamt-N bei 1000g Frischgewicht des Materials anbetrifft, so ist, obwohl in Tabelle XXXII nicht die absoluten Werte, sondern nur die Zu- oder Abnahme gegen die Kontrollwerte angegeben worden sind, zu erkennen, dass er im allgemeinen bei der Blattspreite am grössten und drei bis vierfach so gross wie der bei Blattstiel und Stengel einschliesslich der betreffenden Knoten ist, und beträgt 0.2% des Frischgewichtes. Unter diesem Gesamt-N macht der absoluten Gehalt des Eiweiss-N bei der Blattspreite einen ansehnlichen Teil und zwar 86% des Gesamt-N aus, während er beim Blattstiel und dem Stengel 59.7 bzw. 66.1%, was aber, aus den absoluten Wert berechnet, nur 17% bzw. 22% zum bei der Blattspreite entspricht. Wenn man aber die

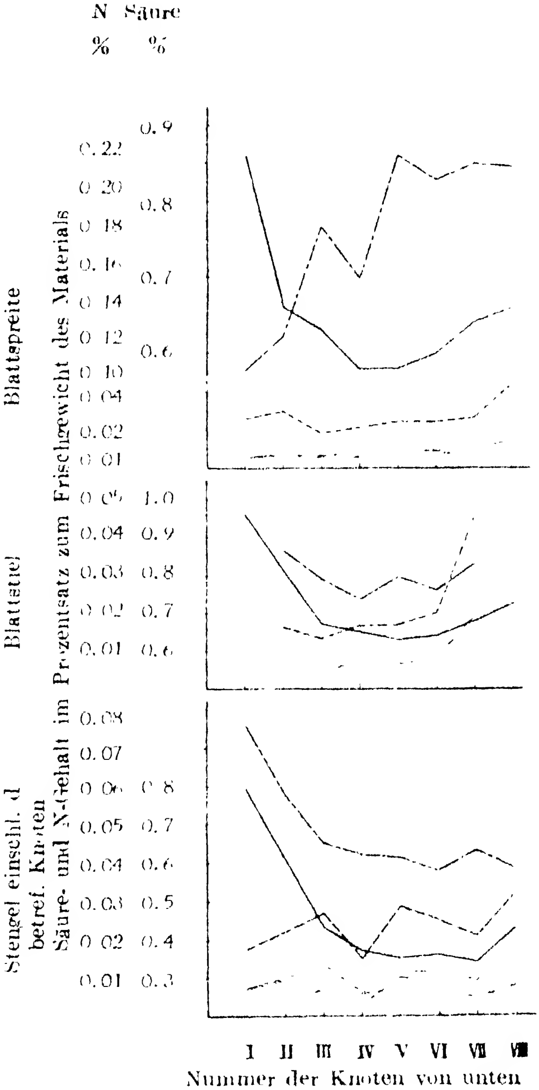

Fig. 11 Säure- und N-Gehalt von *Begonia Evansiana* Andr. (Verdunklungsversuch).

Tabelle XXXII. Der Einfluss des Kohlenhydratmangels auf den Säure- und N-Stoffwechsel. —Verdunklungsversuch (1934)

Pflanzenteil		Blattspreite	Blattstiel	Stengel	im ganzen Pflanzenkörper
mg Oxalsäure in 100 g Frischgew.		−3135.5	−3355.4	+15.9	−2158.3
mg N in 100 g Frischgew.	Gesamt-N	+107.4	−185.2	−115.4	−64.4
	Eiweiss-N	+95.5	−79.7	−99.7	−24.6
	lösl.-N	+11.9	−105.5	−15.7	−36.4
	Ammon-N	−16.2	−12.5	+4.8	−8.0
	Amid-N	+14.2	−66.9	−28.4	−27.0
	Amino-N	+13.9	−26.2	+7.9	−1.5
Amid/Ammon		1.64	0.86	0.67	

einzelnen N-Fraktionen im Vergleich zu den Kontrollwerten betrachtet, so sind die Schwankungen im allgemeinen auffallend wenig, und bloss am löslichen bzw. gesamten N mässigen Abnahme zu erkennen. Der Gehalt an löslichem N in verschiedenen Pflanzenteilen zeigt keine merkwürdige Differenz zum der Kontrollpflanze gegenüber, mit einer Ausnahme beim Blattstiel, wo er zum 2/3 des Kontrollwertes abgefallen ist. Unter den löslichen N-Fraktionen liegt der Ammon- und Amino-N wenig, und der Amid-N, besonders am Blattstiel, etwas grösser zum Abfall unter. Der Quotient Amid/Ammon beträgt 0.39–2.69. Wenn nun, um die etwaige Beziehung zwischen Säurebildung und Eiweisstoffwechsel einzublicken, die absoluten Säuremenge der jeden einzelnen Pflanzenteilen auf dem Ordinat, und der entsprechenden absoluten Eiweiss- oder löslichen N-Menge auf dem Abzisse entgegen kurvenmässig darstellt wird, so nimmt hier der Kurvenverlauf bei den jeden betreffenden Pflanzenteilen so gut wie vollkommen zu dem des CO_2-freien Versuches überein. Und zwar sind die Schwankung der Säuremenge zu den der Eiweiss- oder löslichen N Menge fast überall unabhängig von einander oder etwa negativ korreliert, wie beim CO_2-freien Versuche auch der Fall war. Nur es macht ein Ausnahme beim Stengel, wo die Säure- und Eiweiss-N-Menge positiv korreliert verändert, was aber bei den Kontroll- und CO_2-freien Versuche ebenso der Fall war, sodass es auf die an den damaligen Pflanzen untergelegenen gemeinschaftlichen inneren Bedingungen zurückgeführt werden muss. Wenn man die genannte Beziehung am ganzen Pflanzenkörper von betreffenden Durchschnittwerten aus betrachtet, so ist die Säuremenge zum 3/4 von Kontroll- und zum 9/10 von CO_2-freien Versuchen abgefallen. Diese Ergebnisse stimmen mit den vorjährigen Versuchen 27 und 28 insofern gut überein, dass die

Dunkelversuche immer das Säureniveau niedriger als die CO_2-freien Versuche gaben. Trotzdem ist kein Anhaltpunkt über die Grundlage der Säurebildung gegebn, eben weil die Abbauprozesse hier so stark waren, dass die in Betracht zu ziehenden Stoffe ausnahmslos zur Abnahme beeinträchtigt wurden.

(Fortsetzung folgt.)

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ON A FACTOR AFFECTING THE VELOCITY OF EXCITATORY CONDUCTION IN THE PETIOLE OF *MIMOSA PUDICA*

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(With 4 Text-figures)

In the main petiole of *Mimosa pudica*, moderate conduction of excitation (*m*-wave) propagates through the whole of it with a constant velocity, though in some cases acceleration or retardation may be observed (SIBAOKA 1950b). When a small portion of the main petiole is half cut across to remove some parts of the conductory tissue, the velocity of conduction decreases at this portion, but increases in the succeeding intact portion to take up the original velocity. The velocities of conduction in the part of one half of a vertical split of the main petiole are less than those of the normal one. In the case of weak stimulation, the conduction is slow, but at some distance from the stimulating point it accelerates to recover the constant velocity In the sub-petiole, on the other hand, the excitation is not conducted at a constant rate, but has a higher velocity in the more basal part.

In the present paper are described the new results concerning the acceleration or the retardation of the excitatory conduction in the main and sub-petioles. The velocities of conduction are determined from the oscillogramm of action potentials, as in the previous experiments (SIBAOKA 1950b).

1. *Acceleration and Retardation of Conduction in the Sub-petiole in the Normal Condition.*

The action potential of the sub-petiole (the pinna-rachis) shows some additional small deflections due to the excitations of the fork of every pair of laminar pulvini, between the two leading electrodes, so that the velocities between all of any two pairs of laminar pulvini may be calculated from their oscillogramms. A pair of leading electrodes connect the apical-most leaflet with the basal-most one, as is shown at the bottom of Fig. 2. When a point of S_1 or S_2 (Fig. 2) was stimulated by cooling water, the action potential can be lead off in the sub-petiole (Fig. 1), not in the leaflets, as the moderate conduction does not pass through pulvini, so that the excitatory conduction throughout almost all parts of the sub-petiole can be known by this method.

As previously reported (SIBAOKA 1950a), when basipetal conduction is induced by a cooling stimulus at S_1, the intervals of small deflections become less and less (Fig. 1, *A*), while in the case of acropetal conduction caused by the stimulus at S_2 the reverse is true (Fig. 1, *B*). This difference may suggest that accelerative conduction occurs in the former case but is retardative in the latter. The conduction velocities in every part between any two pairs of laminar pulvini are shown on the top of Fig. 2, averaged from the values of three experiments in both directions on a sub-petiole. In this figure, the early stages of both directions were not indicated due to the obscurity caused by technical disturbance at the beginning of the action potential or to the small deflections occurring in its rising phase. With the exception of cases of good condition and of sufficient stimulus, the excitation by cooling stimulus (*i. e.* supplying about 1°C. ice water) is not generally conducted through the whole sub-petiole. In the cases of heating or cutting stimulus, on the other hand, the small deflections are disturbed by the *s*-wave (*cf.* SIBAOKA 1950a) so that the calculations of velocities in each parts of the sub-petiole are difficult. UMRATH (1928, 1937) also studied the action potentials in the sub-petiole, but because

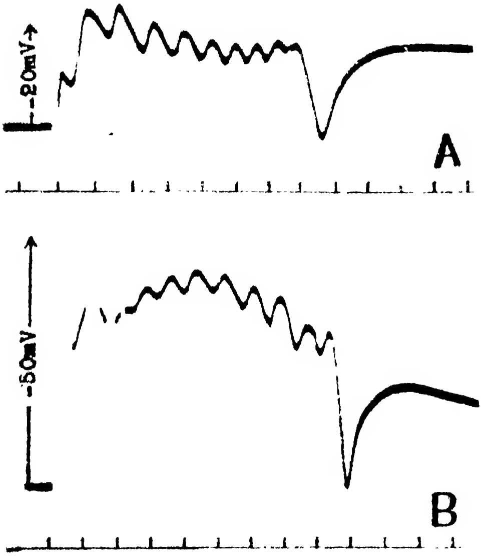

Fig. 1. Curves of action potential in the sub-petiole, *A* : basipetal conduction. *B*: acropetal one. Time marks 1 sec apart.

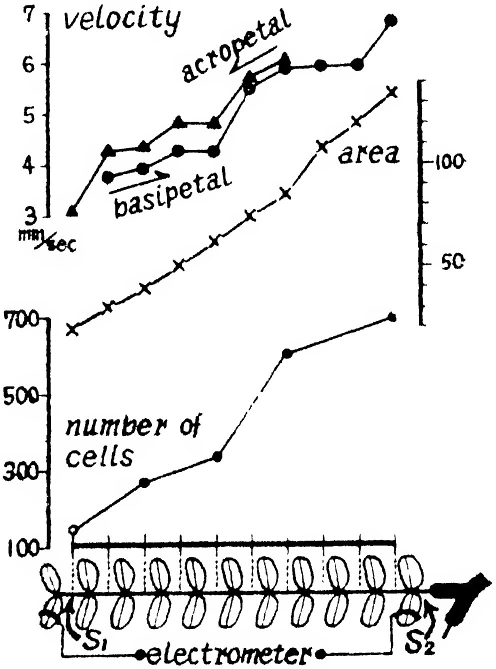

Fig. 2. At the top, the velocities of conduction. At the middle, areas and cell numbers in the cross section of main central cylinder on each parts between leaflet pairs. At the bottom, the leading circuit of the action potential, appointing stimulating points (S_1 and S_2).

The scale of area indicates the reading of planimeter, 100 correspond to about 0.02mm^3.

of his incomplete recording apparatus he overlooked the small deflections in the *s*-wave and called them "Aktionsstromgruppe". In the sub-petiole of *M. Spegazzinii*, SNOW (1925) found from the degree of reaction of a leaflet, that in the cases of besipetal and sometimes in acropetal conduction the acceleration of conduction velocity occurs by cutting stimulus, but without retardation.

The top of Fig. 2 shows the following facts: In general, there is no difference between the velocities of basipetal and acropetal conduction in each parts; in other words, each parts have a proper velocity without reference to the direction. The acceleration in basipetal conduction and the retardation in acropetal, results from the arrangement of those parts having proper velocities which increase from top to base.

The writer previously suggested (SIBAOKA 1950b), that there are several numbers of excitable units in the petiole which may be pointed out in cross section, and if the unit quantity is decreased in some way the conduction velocity also decreases. After the oscillographic record has been taken, the areas in cross sections of the middle parts between each two pairs of laminar pulvini are measured. All of the areas in cross section of the whole, the cortex (inclusive of a ridge bundle) and the main central cylinder increase from top to base in strong correlation with the conduction velocity of each corresponding parts (Fig. 2). The correlation coefficients in these three cases are $r = 0.93$, $r = 0.92$ and $r = 0.94$ respectively. From testing by the distribution of F they are significant on the level less than $\alpha = 0.001$.

The number of cells in the central cylinder (exclusive of the vessels) and cortex (exclusive of a ridge bundle) which may be counted in cross section also increase in strong correlation with the velocity (Fig. 2). The correlation coefficients between the number of cells and the conduction velocities on the parts concerned are $r = 0.99$ in the former case and $r = 0.98$ in the latter. They are highly significant in the former being on the level less than $\alpha = 0.001$ and in the latter, $0.01 > \alpha > 0.001$.

As described above, the velocities of excitatory conduction in every part of the sub-petiole are also related strongly to the area in cross section, *i. e.* to the number of cells, of these corresponding parts.

2. *Artificial Retardation in the Main Petiole.*

In the main petiole the excitation is conducted through the whole with a constant velocity, but when a portion of it is half cut across, the velocity decreases only at that portion. If in similar experiments to the previous one several leading electrodes are set on several positions of the main petiole and connected with one electrometer, it is possible to measure the conduction velocities of each parts either between any two electrodes, or between an electrode and main pulvinus. When a zone over about 5 mm of the main petiole is scraped away to remove the epidermis, cortex and two ridge bundles, so as to leave only the main central cylinder, which is sheathed around by sclerenchymatous layers, and the depth of the wound is afterwards determined under the microscope, it was found that the conduction velocity in this zone does not practically change,

Table I

Individual and leaf No.	Temp. (°C.)	Velocities of conduction (mm/sec)	
		Normal part	Operated part
Mature, II	29	6.2	7.0
Mature, II	29	7.2	7.8

as shown in the table; the *s*-wave normally passes thereafter through this zone But when two ridge bundles and some neighboring parts of the epidermis and cortex still remain intact, we can observe weak conduction of excitation which may be determined by a very small action potential, though it is not conducted to a greater distance. Therefrom, it follows that the conduction of excitation occurs almost in the main central cylinder.

Next, some parts in the main central cylinder of a main petiole are wounded by picking with a platimum-iridium needle (0. 15 mm dia.), to measure the velocity of conduction in this wounded portion in comparison with that of the normal. The needle is inserted perpendicular to the longitudinal axis of the petiole, and in the case of more than two pickings the needles are inserted on the same level as the former. By such operation, we can make various degrees of wound on the same level across the petiole. More than one day after the petiole has been operated as mentioned, two leading electrodes (*B*, *C*) are set 2~3mm above and below the operated point, and the conduction velocities between these two electrodes (*B*—*C*) are measured in comparison with averaged values of *A*—*B* and *C*—*P* (*P* indicates the main pulvinus) (Fig. 3). After taking the oscillographic record the areas of the whole and of non-wounded parts of phloem and of pith

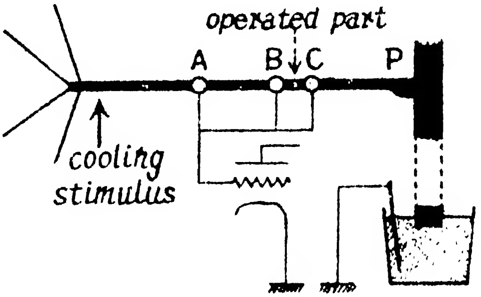

Fig. 3. Diagramm of leading circuit in the experiment on the main petiole (see the text).

in the main central cylinder are measured in cross section under the microscope, and then the ratio of the non-wounded part to the whole is determined.

Sixteen pairs of the relation between the ratio of velocities in the operated part to those of the normal which is the average of three or four measurements, and the ratio of intact areas to the whole are shown in Fig. 4. The larger the intact parts the larger the velocities is, as observed especially in the phloem. The correlation coefficients of these sixteen pairs are $r = 0.61$ in phloem and $r = 0.48$ in the pith. From testings by the distribution of F, the former is recognized as significant on the level of $0.05 > \alpha > 0.10$, but the latter is insignificant.

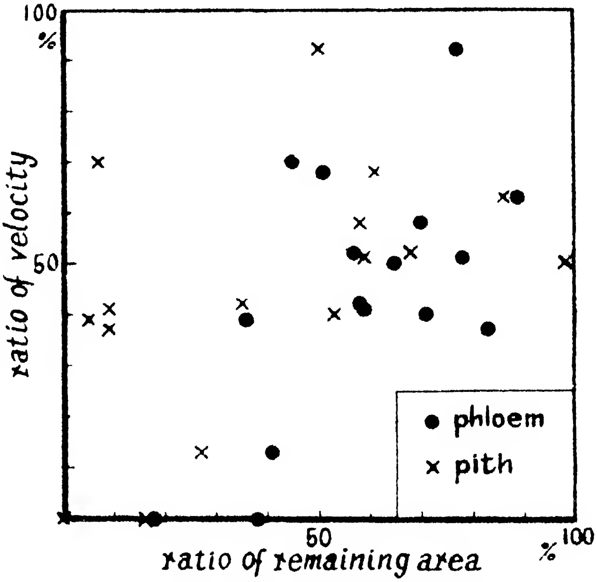

Fig. 4. The relationship between the ratio of velocities in operated part to those of normal portion, and the ratio of the intact area to the whole in phloem and in pith.

From these results it follows that also in the main petiole the velocities of excitatory conduction are affected by the cross area of the petiole and especially of the phloem, so that the path of excitatory conduction lies mainly in the phloem, as already suggested by Herbert (1922), Snow (1924), Bose (1926) and Umrath (1937).

3. *Conclusions.*

The velocities of the moderate conduction of excitation in the main and sub-petiole depend on the sizes of the petiole. Accordingly, in the sub-petiole of which the sizes increases from the top to the base, acceleration occurs in basipetal conduction or retardation in acropetal, while in the main petiole having about constant sizes over their whole length, the excitation is conducted with a constant velocity, and when a small portion is picked off the velocity decreases only in that portion.

The size, *i.e.*, the area in cross section, may be expressed by the number of cells, as strong correlation is found between them and the velocity.

In the main petiole the conduction velocity is affected by the number of cells in the phloem.

As described above, it may be concluded that the excitable units which have been suggested by the writer in a recent paper are some cells belonging to the tissue of phloem.

The writer is indebted to Dr. Y. YAMAGUTI for his supervision during the course of this work.

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THERMOELECTRIC STUDY ON THE SAP STREAMING OF PLANTS

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(With 13 Text-figures)

I. INTRODUCTION

The problem of the sap streaming of plants has been investigated by many researchers. The direct observation of the sap direction, velocity and others has been long observed since STRASBURGER (1891) by many workers as GOPPELSROEDER (1901), HUBER (1932), STRUGGER (1940), and ROUSCHAL (1940). This method is, however, inconvenient, as we can use merely cut plants, and is doubtful whether the measured velocity is in accordance with that of intact plants or not. A pigment may be left behind from ascending water or stored up on the cell membrane. This method is further advanced by STRUGGER in late years, taking advantage of a new pigment "oxypyrentrisulfosaures Na". Recently, however, the new phase of study is opened in this field by HUBER's thermoelectric researches on trees and climbers. In higher animals thermoelectric method of measuring the velocity of flow in the tube, according to HUBER, was first used by H. REIN for the measurement of velocity of blood current. In 1932 HUBER himelf used it for investigating the velocity of the transpiration current. About the same time, DIXON and others applied the thermoelectric method to follow the direction of the flow of liquid in plants. HUBER's thermoelectric procedure (1932) is superior in observance of untouched plant. It allows the measuring of the velocity on different parts at various times repeatedly, and the catching of the partial and temporal distribution of velocity. But HUBER himself was not successful in herbaceous plants. He had in fact investigated the trees but not herbaceous. Later HUBER, SCHMIDT and JAHNEL (1937) experimented, and HUBER and ROUSCHAL (1938) attempted the thermoelectric comprehension of assimilation stream without success.

The present paper reports the results of measuring the velocity of transpiration current in ***Impatiens balsamina*** and some other plants by thermoelectric and "compensation method".

Before proceeding further, I wish to express my sincere thanks to Prof. Dr. Y. YAMAGUTI for his kind instruction in the course of this work.

II. METHOD AND MATERIAL

In the present experiments the thermoelectric method of measuring the velocity of flow in tubes was used. It essentially consists in the local application of heat to the presumed channel of the moving liquid, and the detection of this heat by means of a thermocouple on the channel at a measured distance from the source of heat. The heater in these experiments consisted of a piece of silver wire (0. 1mm. diam.) about 10 cm. long. The wire insulated with varnish was soldered to the ends of copper leads coming from a switch connected to a 2 volt battery.

The thermocouple used were of copper-constantan junction; two insulated copper leads (0. 1 mm. diam.) about 50 cm. long are connected with constantan bridge, and the terminals of the copper leads from the copper elements with the galvanometer, thus reducing as far as possible the number of connections in the circuit, which are apt to introduce error. By the connection of terminals of the leads with a switch, the different couples may be put in circuit with the galvanometer. In order that the unavoidable junctions in the switch may not introduce thermoelectric error, these junctions are kept at the same temperature.

The galvanometer used in this work was a mirror galvanometer with moving coil D-3 type, made by the Yokogawa Electric Works Ltd., having a whole resistance of 17 ohms, and the inner resistance of 7 ohms. Its sensitivity is 1.4×10^{-8} amperes. and free period 5 seconds. In this arrangement a temperature difference between the junctions of 1/100°C. gave a deflection of about 1 division on the scale at the distance of one meter from the galvanometer.

The plant material, *Impatiens balsamina*, is white flowered variety. In spring, the seedlings are taken in pots, cultivated out of doors, and after growing to more than about 30 cm. in height, they are used for the experiments. The experiment room is a dark, semi-basement with almost no circulation of air even when the door and window are opened, and high content of moisture in the air throughout the whole experiment.

A considerable distance is intervened between the heater and the points of detection, so that the junction might not be affected by the heat conducted by the intervening tissues or by the hot air rising from the heater set below the couple. This disposition of the heater and the couple limits the method to the observation of comparatively rapid velocities. For, during the slow passage of the fluid through a considerable length of intervening tissues, the heat gradually will be dissipated, and will not be revealed by the couple concerned.

In HUBER's and BAUMGARTNER's experiments on the transpiration current, distances of 4 cm. to 25 cm. intervened between the source and the couple, but as they were dealing with velocities of 5 cm. to 30 cm. per minute, this showed no disadvantage. Where velocities of 1 or 2 cm. per minute are concerned all of the heat applied will be lost. In reducing the distance, however, there is a danger that the heat conducted through the tissues may lead to raise the temperature of the nearer junction of the couple, and this may be confused with the looked for rise due to the moving liquid.

The heater and thermocouples were set at heights 15–20 cm. above the surface of the earth in the pot, and the distances between the heater and thermocouples varied from 6–15 mm.

The thermocouples affixed to the stem were so arranged that, when the junction nearer to the heater was at a higher temperature than the more distant one, the current passing in the circuits caused a positive deflection of the galvanometer. Thus, when the switch of the heater current was closed momentarily (viz., for 2, 3, or 5 secs.), at first a positive deflection was observed. Then, as the heat reached the more distant junction, the deflection declined towards zero; and, if the temperature of the latter junction become higher, the deflection will turn to be negative, and then again to reach to zero.

III. EXPERIMENTAL RESULTS

Experiment A, 1.

The first research was performed from summer to autumn in 1939. The thermocouples are arranged on the down-stream side of the heater, distance 10 mm. intervened between the heater and the first junction of thermocouples, and the second junction 10 mm. apart more on the same side (Fig. 1).

In Table I the deflections of galvanometer recorded every 10 seconds from the moment of heat application are given. These results are also visualized in Fig. 2.

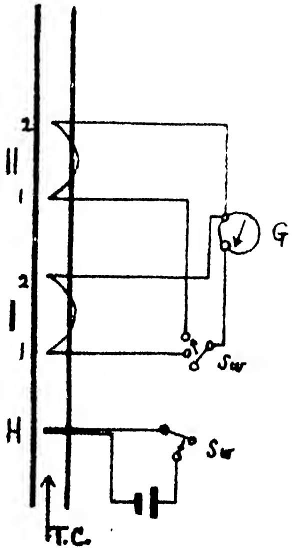


Fig. 1. Scheme showing the arrangement of the apparatus.

T. C. = Direction of the transpiration current; H. = Heater; I, II. = Thermocouple; 1, 2. = Junction; Sw. = Switch; G. = Mirror galvanometer.

Table I. *Impatiens balsamina*. (3. X. 1939)

Time	Galvanometer deflection (scale division)	Time	Galvanometer deflection (scale division)
12.47 0 sec.	0	15.50 0 sec.	−0.1
9.8	beginning	10	−0.1
19.5	2.80	20	−0.1
30	0.20	24.5	beginning
40.5	−1.00	44.5	3.70
50	−0.30	50	2.60
60	0	60	−0.70
		70	−1.80
		80	−0.90
		90	−0.30

Table II. *Impatiens balsamina*

Date and time		Measuring distance	Beginning of the first deflection of galvanometer after sec.	Velocity	
				m./h.	cm./min.
X 3	15.50	II I$_1$ 10mm	24.5	1.46	2.44
X 3	12.47	10	9.8	3.67	6.12
IX 26	12.05	20	21.0	3.43	5.71
X 12	14.20	15	14.5	3.72	6.20
X 12	14.40	15	12.5	4.32	7.20
X 12	15.30	15	18.0	3.0	5.0
VIII 8	9.00	15	30.0	1.80	3.00
	11.30	15	28.1	1.90	3.18
	15.00	15	32.0	1.65	2.76
VIII 12	10.55	15	7.5	7.2	12.0
	12.30	15	8.0	6.7	11.2
VII 12	9.30	12	38.0	1.11	1.86
	13.30	12	40.0	1.08	1.80
	15.00	12	45.0	0.95	1.59
	18.00	12	47.0	0.91	1.53
VII 2	14.10	12	11.0	3.92	6.54
VII 9	10.40	12	24.0	1.80	3.0
VIII 23	10.00	12	45.0	0.95	1.59 +
	11.20	12	42.0	1.02	1.71 +
VIII 17	12.00	12	25.0	1.72	2.88
	12.30	12	26.0	1.65	2.76
	13.00	12	25.5	1.69	2.82
VIII 25	13.00	12	28.0	1.53	2.56
	14.30	12	27.0	1.59	2.66

+ Visualized in Fig. 3: VIII 23, 1 and 2.

The Table II gives a record of some similar experiments, showing the velocities of the streaming on some different dates.

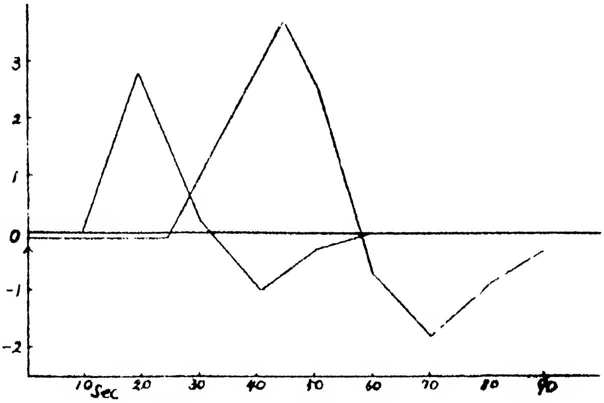


Fig. 2. Graphs showing the deflections of galvanometer caused by the flow of heated water. The zero in the abscissa indicates the moment of the application of heat.

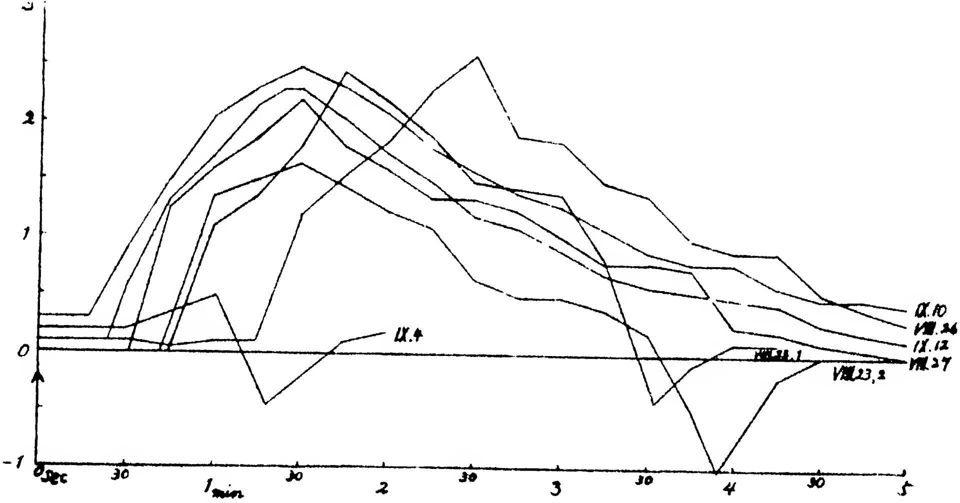


Fig. 3. Graphs showing the various deflections of galvanometer in *Impatiens*. Among others IX 4, VIII 23, 1 and 2 represent the movement of heated liquid.

Table III. *Clematis ternifolia*

Time		Measuring distance	Beginning of galvanometer deflection after sec.	Velocity	
				m./h.	cm min.
X 12	11.00	12mm	43	1.00	1.67 +
	12.20	12	47	0.91	1.53 +
	13.30	12	51	0.84	1.41
	13.40	12	54	0.79	1.33
	13.50	12	55	0.78	1.30
	14.30	12	54	0.79	1.33
X 13	11.45	12	42	1.02	1.71 +
	15.00	12	42	1.02	1.71 +
	15.15	12	43	1.00	1.67 +

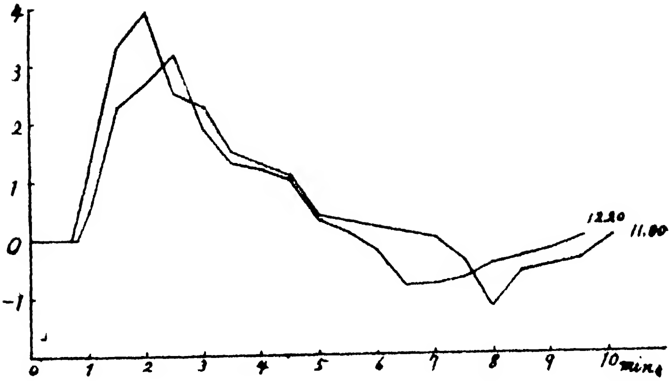
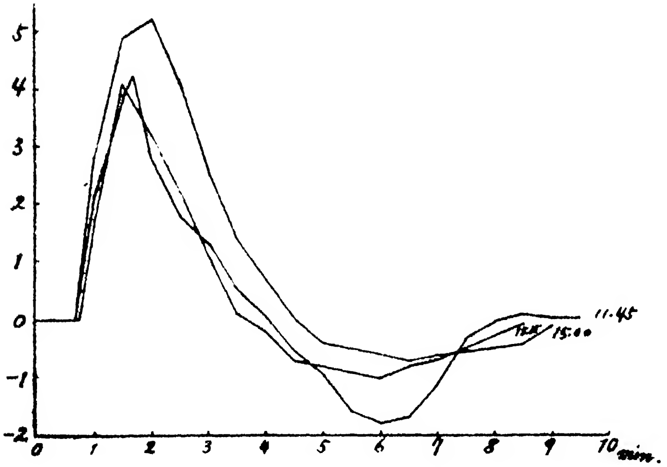


Fig. 4. *Clematis ternifolia*, deflections of galvanometer in scale division. Oct. 12.



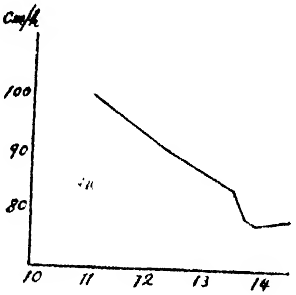


Fig. 5. *Clematis ternifolia*, deflections of galvanometer in scale division. Oct. 13.

Fig. 6. *Clematis ternifolia*, diurnal change of velocity of transpiration streaming on Oct. 12.

Table IV. Diurnal change of velocity of streaming in *Impatiens balsamina*

Time		Velocity	
		m./h.	cm./min.
VIII 12	6.00	1.38	2.3
	7.20	1.68	2.8
	8.30	1 80	3.0
	9.40	3.24	5.4
	10.55	7 20	12.0
	12.30	6.72	11.2
	15.00	3.72	6.2
	16.30	3.00	5.0
	17.30	2.10	3.5
	19.00	1.68	2.8

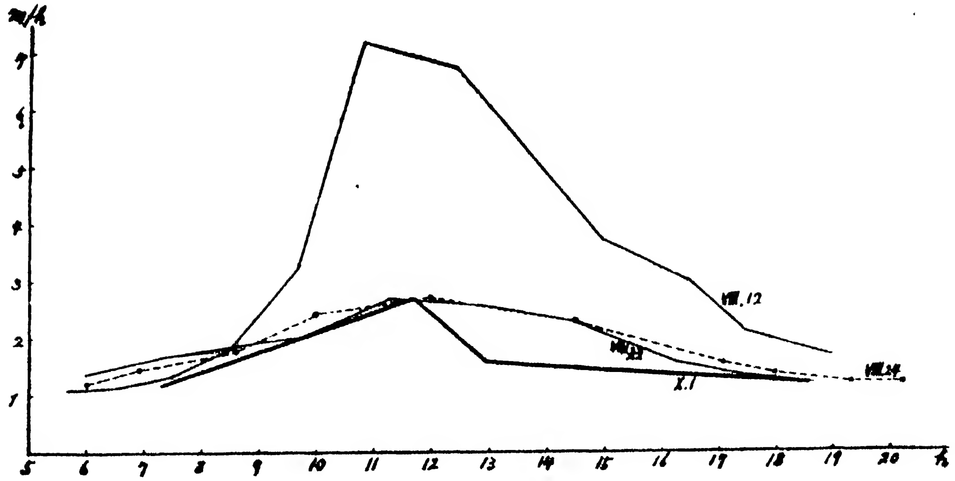


Fig. 7. Diurnal change of velocity in *Impatiens*, VIII 12; and in *Fraxinus*, VIII 24, 22, X 1.

Table V. *Fraxinus*; diurnal change of velocity of streaming

Time			Velocity	
			m./h.	cm./min.
VIII	22	5.40	1.08	1.8
		6.35	1.14	1.9
		7.30	1.38	2.3
		8.35	1.86	3.1
		9.52	2.04	3.4
		11.20	2.70	4.5
		12.51	2.58	4.3
		14.30	2.28	3.8
		16.15	1.56	2.6
		17.30	1.32	2.2
		18.34	1.20	2.0
VIII	24	6.00	1.20	2.0
		6.57	1.44	2.4
		8.40	1.80	3.0
		10.00	2.40	4.0
		12.00	2.64	4.4
		14.30	2.28	3.8
		17.05	1.56	2.6
		18.00	1.38	2.3
		19.20	1.20	2.0
		20.10	1.20	2.0
X	1	7.20	1.20	2.0
		11.40	2.70	4.5
		13.00	1.56	2.6
		18.30	1.20	2.0

These results indicate the occurring of two different types. In the first (Type I) the positive deflection declines toward the zero line, but do not reach the starting level. In the second (Type II), it not only reaches this level but actually pass it. Moreover, some variations occur in both types.

With regard to Type I:

(a) The curve in its return from the maximum positive deflection almost reaches the starting level, but does not pass it, running practically along the starting level. It is assumed to show no convection. (Fig. 3; IX 12.)

(b) The curve in its return from the maximum positive deflection does not attain the original level. (Fig. 3; VIII 26.)

These inflections in some cases may indicate the arrival of a certain amount of heat transported by a moving liquid, which, however, is not sufficient, owing to dissipation, to bring the curve back to the starting level. The record of such experiments are not included in the table (e. g. curves IX 10, IX 12, VIII 26, VIII 27 shown in Fig. 3).

With regard to Type II:

(a) The dip below the starting level on the declining arm of the curve is gradual, as is also the case in its climbing back again to the original level. (Fig. 3; VIII 23, 1 and 2. Fig. 4.)

It is probably due to the gradual spreading of heat in the moving metarial or into the surrounding tissues, as the result of slow movement of the heated liquid.

(b) The recovery from the first deflection, and the turning from the negative excurtion to the starting level are very rapid. (Fig. 3; IX 4.)

It appears to be associated with a rapid movement of heated material, which traverses the distance between the heater and the distant junction so quickly that little spread of heat has occurred in the moving substance or into the adjacent tissues. As was pointed out before, the Second Type of curve indicates that heat has been transmitted to the more distant junction, granted that external disturbances are eliminated. When Type II is encountered we may be sure that there has been realized the transportation of heat, and hence the movement of material in the stem.

Experiment A, 2.

Where the two couples, one above the heater and one below, were supplying records, two results are obtained in experiments concerned. In these experiments upward and downward movements were both recorded as positive deflection. The rapid movement upwards shown in the experiments indicates that the transpiration current in the stem provides a vehicle for the upward tranmission of the heat.

The question as to the tissues which provide the channels for the moving matter naturally obtrudes itself. The very rapid movement upwards shown in the experiments, however, indicates that the transpiration current in the wood provides a vehicle for the upward transmission of the heat. This view has been confirmed by a few experiments, where extirpation of the bark above the heater did not markedly diminish the downward sweep of the recording curve below the starting level. Hence, with suitable reservations, we may at present assume that part of the curve records the transportation of heat in the transpiration current.

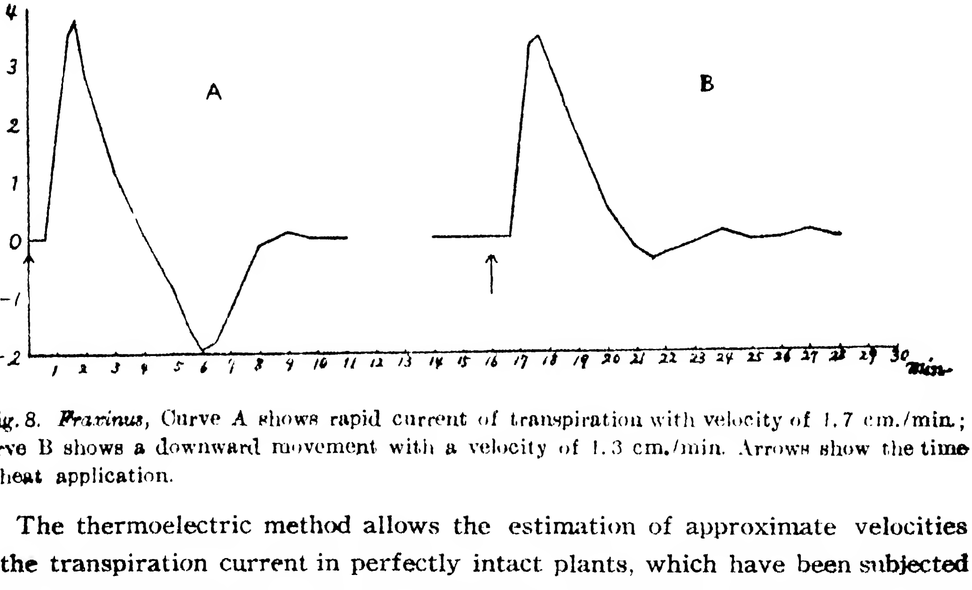


Fig. 8. *Fraxinus*, Curve A shows rapid current of transpiration with velocity of 1.7 cm./min.; Curve B shows a downward movement with a velocity of 1.3 cm./min. Arrows show the time of heat application.

The thermoelectric method allows the estimation of approximate velocities of the transpiration current in perfectly intact plants, which have been subjected to no organic interference. A few experiments made do not contradict the view that the movement has taken place in the vessel.

It has generally been considered (until recently) that mineral nutrients absorbed from the soil were carried upwards in solution in the water of transpiration current, passing through the xylem, and the material elavorated in the leaves passes down through the phloem of the bark. Curtis concludes that the ringing experiments offer strong evidence that nutrients are chiefly carried through the phloem.

Attempts of many sorts were made by Birch-Hirschfeld to bring about a rapid movement of lithium nitrate or eosin through phloem and parenchyma tissues. When lithium salts or eosin were introduced into the xylem, they were quickly carried through the plant, not only toward the transpiring leaves but also backwards to leafless parts. Since the phloem seems incapable of carrying solutes at the necessary rates, whereas the xylem carries introduced solutions with great rapidity in both directions, she suggested that it is possible that the xylem is the normal path of backward transport.

DIXON and his associates supported the suggestion that a backward transport through the xylem is normal. KASTENS considered that foods are carried in both directions through the xylem and not the phloem, and that the disturbed growth responses following ringing are to be explained on the grounds that special hormones controlling behavior are transported through the phloem. MACDOUGAL, as a result of anatomical and injection studies, concludes that in conifers water with its contained solutes rises in the inner layers of the wood, and that possibly sugars are carried downward in the outermost layers of wood, which are not directly connected anatomically with the leaves. ARNDT, as a result of injection experiments, found the rapid movement of eosin both upwards and downwards through the outer xylem layers, even when all effects due to unequal gas pressure, capillarity, and saturation deficits were supposedly eliminated. This led him to suggest that the xylem is normally concerned in transport in both directions, but that the mechanism involved is unknown.

Experiment B.

In the case of such experiments mentioned above I should have taken into consideration the effects of heat conduction. Of course, on lying the stem horizontally the effects of convection may be eliminated, but heat conduction, specially in the slow streaming, may cause a serious error.

Hitherto, HUBER (1932), BAUMGARTNER (1934), HUBER and SCHMIDT (1936), DIXON (1937), ROUSCHAL (1939) and others had reported the thermoelectric measuring of the sap streaming, where measuring distances from heater to thermojunction were 4 cm. or more apart. After HUBER, 4 cm. distance between the heater and thermojunctions in the ordinary thermoelectric method is unsuitable to measure the slow movement of sap about 1 cm. per min. (60 cm./h.) or slower. In reducing the distance, on the other hand, there will arise a danger that the heat conducted directly through the tissues may lead to rise the temperature of junction of couple, and this may be confused with the looked for temperature by moving liquid. For that reason it has been taken the compensation arrangement by HUBER and SCHMIDT (1937), setting the under junction nearer to the heater the upper one (e.g. 17 : 20 mm., or 8 : 15 mm.; ref. Fig. 9.)

As the conduction of heat through the tissues is spreading uniformly on both sides by the law of fall of temperature, the under junction being arranged nearer gains over the temperature projection against the upper junction after some seconds on heat application, and causes a deflection of galvanometer to the left in the case where the streaming is not in existence. The heat will pass over and diminish in tissues. The upper junction being more distant becomes warm later than the under one, so that the temperature difference between two junctions will be gradually eliminated and the galvanometer deflection towards right be increased in magnitude till the maximum is attained, and be back again to zero, finally.

Now in living plants, due to the transpiration streaming in vessels, the upper junction must touch earlier than the under one with the ascending warm wave, being the combination of heat conduction and streaming. The heat conveyed to the upper junction (down stream) by the moving liquid has to change the direction of deflection from left towards right as in Fig. 10, finally returning to zero, when the heated liquid has passed. In this case an early turn of galvanometer deflection from left to right must be observed as compared with the former case (stream is not in existence).

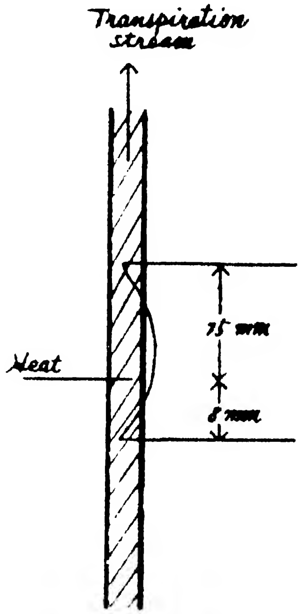
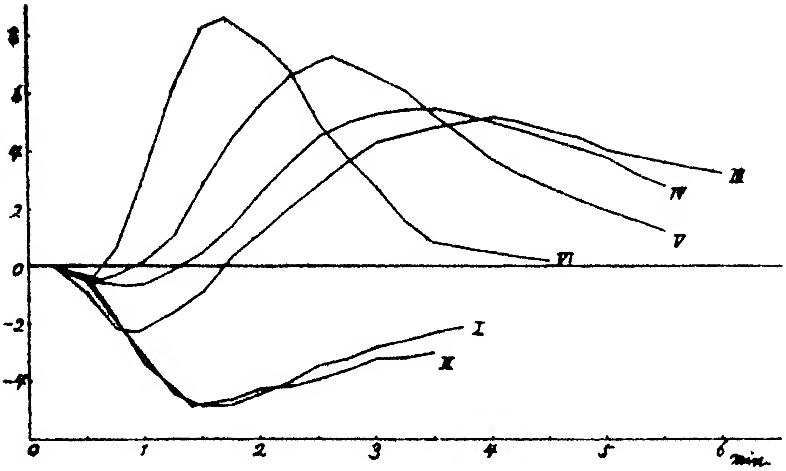


Fig. 9 Arrangement of thermocouples in compensating principle.

The time of early turn of deflection is a measure for the velocity of transpiration streaming. When the velocity of streaming compared with conduction of heat is so rapid, the deflection of galvanometer lean to the right directly.

The limit of sensitiveness of this method after Huber is enough to measure the velocity of slow movement about 1 mm./min., (5cm·/h). The absolute accuracy of this method depends on the determination of turning point in the case of no streaming (under the control of transpiration). The relation between the velocity of streaming and the time needed for the turning of galvanometer deflection in the compensation method is formulated as follows:

In the case of transpirating plant, the time interval in sec. from the heat application up to the deflection turning (under the base line) is put t which is liable to change by the velocity of streaming. The corresponding time interval in sec. in the case of conducted heat, which may really occur by the suspension of transpiration by means of raining upon or by cutting off the stem, is put t_0. The calculation of velocity of streaming (V_k) is carried out by the formula



	Under turning point	Upper turning point
	min. sec.	min. sec.
I	1 30.0	- - -
II	1 26.0	—
III	58.0	4 0.6
IV	48.0	3 24.2
V	36.0	2 42.6
VI	34.2	1 42.4

Fig. 10. Under curve, no streaming; above, with streaming.

$$s = t(V_k + V_1)$$

$$V_k = \frac{s}{t} - V_1$$

$$V_1 = \frac{s}{t_0}$$

$s = 20$ mm. distance.

V_1 = Velocity of pure heat conduction, constant value for definite plant individual.

Then

$$V_k = \frac{s}{t} - \frac{s}{t_0} \text{ cm./sec.}$$

The time of the first turning point of deflection depends naturally on the velocity of streaming and is proportional to it, so that in the case of a very slow movement of the stream, t will come to coincide with t_0.

Among others, the heat intensity has almost no effect on the measurement of streaming velocity (in the case of heating duration 3-6 sec.), while the conduction of heat depends on the heat intensity.

Measurment of t_0:— s/t_0 is the expression of heat conduction and has the constant value for definite plant tissue. To measure the value t_0 the streaming is stopped artificially by cutting the stem tip about 20 cm. long and applying with vaseline on the cut surface. Thermocouples are arranged at the distances of 6 and 10 mm. from heater on both sides of it as indicated above (Fig. 9).

Table VI. Galvanometer defliction in scale division, *Impatiens*

Time after heating		Example 1	Time after heating		Example 2	Time after heating		Example 3
min.	sec.		min.	sec.		min.	sec.	
	0	0.00		0	0.00		0	0.00
	15	−0.30		15	−0.40		15	−0.40
	30	−2.80		30	−2.30		30	−2.10
	45	−6.00		45	−4.60		45	−4.30
1	0	−6.60	1	0	−6.20	1	0	−5.50
1	7.5	−6.75	1	8	−6.60	1	10.5	−5.90
1	15	−6.40	1	15	−6.30	1	15	−5.80
1	30	−5.30	1	30	−5.60	1	30	−5.40
1	45	−4.40	1	45	−5.00	1	45	−5.10
2	0	−3.90	2	0	−4.20	2	0	−4.10
2	15	−3.30	2	15	−3.55	2	15	−2.80
2	30	−2.85	2	30	−3.10	2	30	−2.20
2	45	−2.35	2	45	−3.00	2	45	−2.10
3	0	−2.00	3	0	−2.30	3	0	−1.85
3	15	−1.60	3	15	−2.10	3	15	−1.70
3	30	−1.35	3	30	−1.70	3	30	−1.50
3	45	−1.00	3	45	−1.40	3	45	−1.30
4	0	−0.85	4	0	−1.40	4	0	−1.10

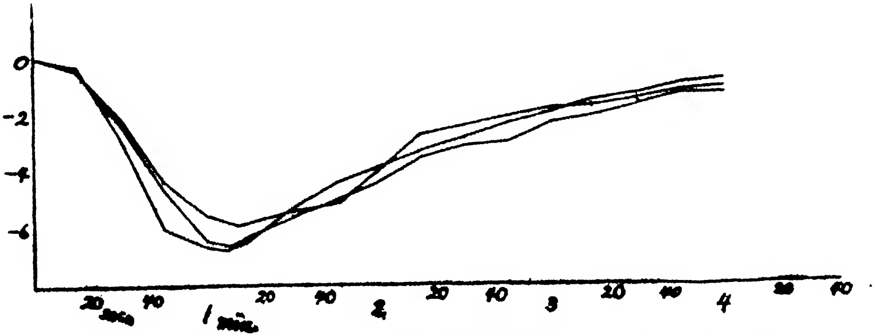


Fig. 11. Deflection of galvanometer for the determination of t_0 in *Impatiens*.

The galvanometer deflections are observed in every 15 sec. from heat application, and the first turning point is sought. Some examples are given in Table VI, and visualized in Fig. 11.

t_0 is found to be 67.5 sec. − 70.5 sec., and deflections return to zoro in about five minutes.

Then, $\frac{s}{t_0} = \frac{10}{67.5} = 0.1481$ mm./sec.

or 8.886 cm./min.

or 53.31 cm./h. in maximum

$\frac{s}{t_0} = \frac{10}{70.5} = 0.1418$ mm./sec.

or 8.505 cm./min.

or 51.04 cm./h. in minimum.

Average 8.742 mm./min.

It is also determined that no deflection may be observed in the case, where the junction distances on both sides from the heater are taken equal. When the streaming is in existence, however, the deflection run towards one side only by the heat brought with the stream, so that the time at the beginning of deflection from heat application is a number concerned with velocity, compensating the effect of heat conduction. Some results are given in Tab. VII and shown in Fig. 12.

Table VII. *Impatiens*

Time	Distance of junction on both sides of heater	Beginning of galv. deflection after	Velocity of stream
X 11, 14.10	15 mm.	19.0 sec.	4.73 cm/min
X 11, 14.45	15	15.5	5.80
X 11, 15.06	15	16.0	5.62

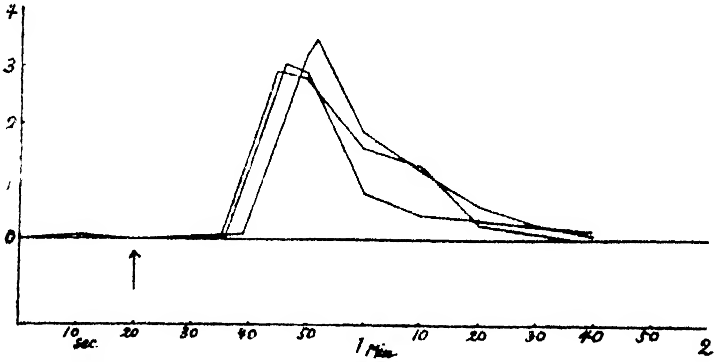


Fig. 12. Showing the galv. deflections of Tab. VII.

The comparative study of velocity of streaming and of heat conduction by the compensation method, taking the up-stream junction nearer than down-stream one has shown that, if the both velocities are equal or the streaming is slower than the conduction, the heat may first be conducted to nearer up-stream junction and cause a deflection to the negative direction, meanwhile the heat transmission by the stream induces thereafter the deflection to turn beyond zero to the positive direction. In fact, however, the velocity of streaming is faster than the conduction of heat, so that the deflection is from the onset to positive direction due to the heat transmission by moving liquids, and then it returns to zero, followed by negative deflection due to the conducted heat, as shown in Fig. 13.

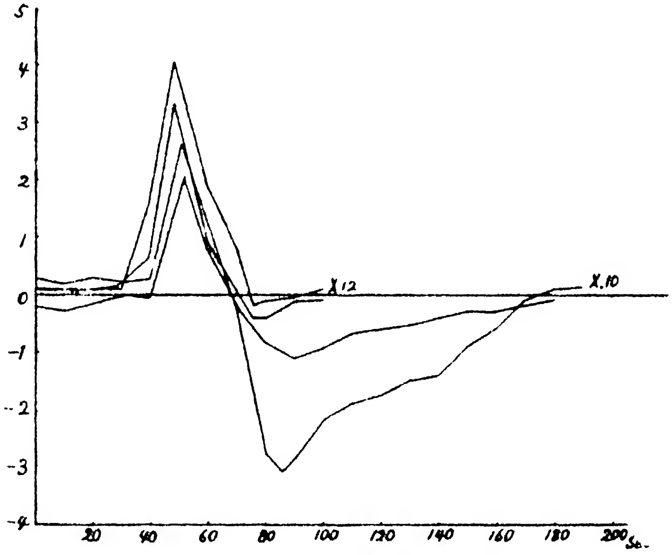


Fig. 13.

The calculations of the velocity in the case of *Impatiens* by the formula $V_k = \frac{s}{t} - \frac{s}{t_0}$, where the $\frac{s}{t_0}$ = conduction of heat = 8.74 mm./min., are given in Tab. VIII.

Table VIII

$\frac{s}{t}$	$\frac{s}{t} \times 60$	Corrected velocity cm./min.
0.540	3.24 cm./min.	2.366 (1.41 m./h.)
0.526	3.15 cm./min.	2.282 (1.36 m./h.)
1.25	7.50 cm./min.	6.626 (3.97 m./h.)

SUMMARY

1. The writer has studied on the velocity of transpiration stream of *Impatiens* and some other plants with thermocouples. The thermocouples were made of constantan and copper elements with enough sensibility to measure the velocity.

2. The general velocity in *Impatiens* is 3–7 m./h., in *Clematis* 1.02 m./h., in *Fraxinus* 2.7 m./h. in maximum.

3. The upward and downward movements were recorded so as to give the positive deflection from which it was established that the xylem is normally concerned in transport to both directions, but that the mechanism involved is unknown.

4. The compensation method (Huber and Schmidt 1937) is tried to measure the velocity in the case of slow movement, calculating the velocity by formula,

$$V_k = \frac{s}{t} - \frac{s}{t_0},$$

where the s/t_0 is the conduction of heat and generally gives the constant value for a definite material. In *Impatiens* $s/t_0 = 8.74$ mm./min. in average. The velocity found in normal condition must be corrected by s/t_0.

5. The compensation method is approved for slow movement, being that the galvanometer deflections differ in the slow streaming compared with fast one. The experiments were also made in the extreme case where no streaming is provable.

6. On the assimilation stream it is not yet proved, but it seems to be possible.

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SYMBOLAE ITEOLOGICAE X[1]

AUCTORE

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(Cum 2 figuris in textu)

117) × **Salix Yuhkii**[2] KIMURA hyb. nov. (Fig. 1).
= *Salix Babylonica* L. var. *Lavallei* DODE × *S. hondoensis* KOIDZUMI.

Descr. specim. original.: *Arbor* habitu ad *S. Babylonicam* appropinquat, 12 m alta, coma fere rotundata, trunco circiter 50 cm diametiente, cortice sordide cinereo-fusco longitudine irregulariter rimoso. *Rami* ramulique penduli (non recte pendulissimi ut in quibusdam formis *S. Babylonicae*). *Ramuli* gracillimi laeves 40-96(-133) cm longi; hornotini in aestate virides, superne minutissime pubescentes, inferne glabrescentes, annotini glabri nitentes, vetustiores cinerascentes. *Gemmae* amentiferae (primo auctumno visae) oblongo-ovatae vel ovatae apice obtusissimae, latere vix vel obtuse carinatae, virides pubescentes, 5-5.5 mm longae, 2-2.5 mm latae. *Cataphylla* sterilium ramulorum obovato-elliptica vel obovato-oblonga, apice obtusa ad obtusissima, basi cuneata brevissime petiolata, margine integriuscula vel integra vel obsolete serrulata, supra glabra, subtus undique vel secus costam adpresse villoso-sericea, prima 9.5-13×4.5-6 mm, proxima 11-16.5×4.8-6.3 mm magna. *Folia recentissima* sub vernatione convoluta, utrinque albo-sericea. *Folia adulta* chartacea, interstitiis 0.8-2.3 cm longis dissita, inferiora minora lanceolata infra medium latiora, suprema nonnumquam lineari-lanceolata (iis *S. Babylonicae* simillima), ceterum plerumque anguste lanceolata vel anguste oblongo-lanceolata, infra medium latiora vel lateribus fere parallelis, apice sensim attenuato-acuminata, basi margine leviter convexo acuta ad obtusissima, margine crenato-serrulata, serraturis in medio folii 4-6 pro 1 cm, supra viridia nitentia, subtus albo-glauca, utrinque primo parce adpresseque pilosa, demum glaberrima, 8.8-13 cm longa, 1.2-2.2(-2.7) cm lata, (4.4-)5.7-6.8(-7.3)-plo longiora quam latiora; costa pallida, supra convexa sub lente pilis minutissimis pubescente (basi densius), infra valde prominente pilis adpressis acroscopicis pubescente demum

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[1] Opusculum hoc est effectum per sumptus "Kagakukenkyuhi" mihi donatos ab Ministerio Educationis pro investigationibus scientiarum.

[2] Nomen hoc dedi in honorem amici amicissimi YOSHIMI YUHKI florae boreali-hondoensis peritissimi auctorisque Florulae Yamagata-Prefecturaeanae.

fere glabrescente; nervis primariis leviter arcuatis, utrinque (infra distinctius) elevatis, utroque latere 18–22, a costa sub angulis 30°–50° proficiscentibus, ante marginem flexuosis adscendentibus proximosque attingentibus, secundariis tenuibus irregularibus cum tertiis anastomosantibus, intermediis 1–3 evolutis. *Petioli* semiteretes, supra canaliculati vel basi sulcata excepta fere plani, pubescentes, infra convexi primo pubescentes demum glabrescentes, 4–10 mm longi. *Stipulae* oblique ovatae vel semicordatae, apice argute acuminatae vel acutae, margine distincte vel obsolete serrulatae, raro integrae 3.0×2.0, 5.3×3.0, 6.8×3.7, 7.5×4.0, 9.5×5.0 mm etc. magnae, supra virides basi glandulis vel paucis vel numerosis praeditae, infra glaucae utrinque pubescentes. *Amenta* ♀ coaetanea, densiflora, longe cylindrica, apice obtusa, recte vel leviter curvula, foliato-pedunculata, 20–28 mm longa, circiter 5 mm crassa; rhachidibus dense pubescentibus; pedunculis 5–9 mm longis, sericeis vel tomentoso-sericeis. *Cataphylla* pedunculi 3–5, inferiora obovato-elliptica, obovato-oblonga, apice obtusissima ad rotundata, superiora oblanceolato-oblonga, apice obtusa vel late acutiuscula, basi cuneata brevissime vel breviter petiolata, margine integerrima vel obsoletissime serrulata, supra glabra, subtus dilute glaucina undique vel secus costam sericea, infima 9–14×4.8–6.5 mm, secunda 13–18×5–6.5 mm, tertia 14–21×6–7.5 mm, quarta et quinta circiter 27–29×8.5 mm magna. *Bracteolae* ellipticae vel ovato-ellipticae, apice truncato-rotundatae vel obtusissimae, intus concavae basi paucipilosa excepta glabrae, extus convexae dimidia superiore parte glabrae, ceterum sericeo-pubescentes, concolores pallide flavo-virides, persistentes, 2–2.5 mm longae, fere 1.2 (in expansione 1.4) mm latae. *Glandula* una ventralis crassiuscula late ovata apice obtusissima, 0.6–0.7 mm longa, 0.5–0.6 mm lata. *Ovaria* viridia ovato-conica, leviter obcompressa, sub-

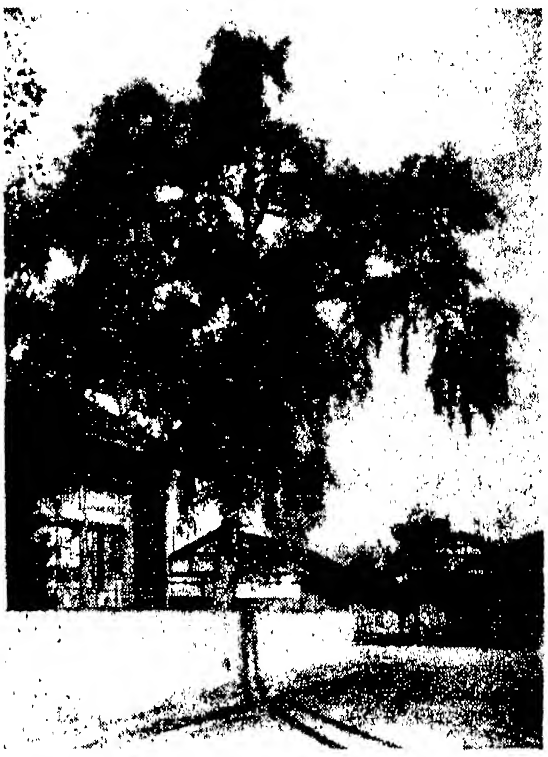

Fig. 1. *Salix Yuhkii* Kimura. Stirps originalis, die 24 Sept. 1949 photographata.

sessilia, dimidia superiore glabra, inferiore albo-sericea, 1.6–2.0 mm longa, 0.8–0.9 mm lata, apicem bracteolae superantia; stylis brevissimis obcompressis 0.2–0.3 mm longis. *Stigmata* anguste cuneata pallide flavo-viridia, apice integra vel emarginata vel bifida, plerumque subcircinatim valde reflexa, 0.8–1.2 mm longa. Ovula in quaque placenta 2.

Nom. Jap. *Yûki-sidare* Kimura nom. nov.

Hab. in Japonia (cult.). Honsyû. Prov. Uzen: Yonezawa, (A. Kimura n. 3046 ♀ fl. [typus] 24 Apr. 1950 in Herb. A. Kimura, fol. [typus fol.] 24 Sept. 1949, fl. 26 Apr. 1949).

Habitu et characteribus inter *S. Babylonicam* var. *Lavallei* et *S. hondoensem* ambigit forsan ex iis hybrida. Ad illam vergens praecipue ramis ramulisque pendulis et stylis brevissimis; ad hanc autem crassitie longitudine et vestimento ramulorum, figura et pilositate cataphyllorum foliorumque plurimorum adultorum, forma et magnitudine amentorum stigmatumque. Foliorum apicis figura et ovaria dimidia inferiore sericea inter parentes fere media. —Non absimilis esse videtur *S. mutsuensi* Koidzumi sed ovariis dimidio superiore glabris, bracteolis extus inferiore sericeo-pubescentibus distinguitur.

118) **Toisusu cardiophylla** (Trautvetter & Meyer) Kimura in Bot. Mag. Tokyo XLII. p. 288 (1928); in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4. p. 396 (1934) (Miyabe & Kudo, Fl. Hokkaido & Saghal. IV.); in Jour. Jap. Bot. XXIV. p. 64 (1949).—Makino & Nemoto, Fl. Jap. ed. 2, p. 175 (1931).—Nemoto, Fl. Jap. Suppl. p. 118 (1936).—Honda, Nom. Pl. Jap. p. 46 (1939).

Syn.[1] *Salix cardiophylla* Trautvetter & Meyer in Middendorff, Reise Sibir. I. pt. 2, Bot. abt. 2. p. 77, t. 19, 20$_{a-i}$ (1856) (Fl. Ochot.).—Nasarov in Komarov, Fl. URSS, V. p. 207 (1936); in Fedtschenko, Fl. Transbaical. p. 193, f. 1–10 (1937).

Ad descriptionem originalem adde: *Amenta* ♂ 3.0–5.0 cm longa, 7–10 mm crassa, rhachidibus parce pubescentibus. Fl. ♂: *Bracteolae* concolores, obovato-orbiculares vel cuneato-obovatae, apice irregulariter undulato-rotundatae, margine ciliatae, extus glabrae, intus concavae fere glabrae vel parce pubescentes, 2.7×2.0, 3.0×2.3, 2.8×1.7, 3.0×1.8 mm etc. magnae. *Glandulae* plerumque 2, ventralis et dorsalis, sed nonnumquam a latere minimae evolutae; ventralis ovata vel ovalis vel obovata, apice rotundata vel truncata, 0.4–0.6 mm longa, 0.3–0.4 mm lata, interdum apice leviter bifida inde circiter 0.6 mm lata; dorsalis oblongo-obovata vel obovata, apice truncato-rotundata, 0.4–0.6 mm longa, 0.3 mm lata. *Stamina* 5–6, si 5 adsunt modo *Choseniae* disposita, filamentis liberis infra fere medium pilosis, 3.6–4.3 mm longis. *Antherae* luteae ovales 0.4–0.8 mm longae.

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[1] De litteratura reliqua confer Kimura op. cit. p. 396 (1934).

Amenta ♀ bracteolis fere deciduis 5.6–11 cm longa, 0.9–1.2 cm crassa, rhachidibus glabris vel minute pubescentibus. Fl. ♀: *Bracteolae* concolores caducae, obovato-ellipticae, apice rotundatae vel truncatae, leviter irregulariterque undulatae, margine parce ciliatae, utrinque glabrae vel intus tantum sparsim pubescentes, 2.6–3.0 mm longae, 1.4–1.6 mm latae. *Glandulae* duae aequiformes, ad basin stipitis ovarii postico-lateraliter sitae, *oblongae vel lineares* (Fig. 2) apice obtusissimae, 0.5–0.8 mm longae. *Ovaria* (tantum inter fructus remanentia visa) ex ovata basi elongato-conica, leviter obcompressa glaberrima, circiter 2.0–2.5 mm longa, 0.7–0.8 mm lata; stipitibus glaberrimis glandulam primo aequantibus deinde superantibus, 0.6–1.0 mm longis. Styli obcompressi bifidi circiter 0.8 mm longi, laciniis circiter 0.5 mm longis. *Stigmata* cum dimidio superiore styli decidua bifida, laciniis linearibus divaricatis 0.3–0.7 mm longis. Capsulae decidendi tempore bracteolarum prope apicem stipitis valde reflexae (quidem sub angulis 65°–90°) maxime ad modum *Choseniae* (Fig. 2).

Nom. Jap. *Karahuto-ôbayanagi* (Miyabe & Miyake, Fl. Saghal. p. 424 an. 1915).

Specimina examinata[1]. Manshuria: inter Ikkenya & Kihsingkou, (M. Tatewaki n. 1768 ♀ 17 Jun. 1943; n. 1771 st. 17 Jun. 1943); inter Kihsingkou & Argun, (M. Tatewaki n. 1824 ♀ 18 Jun. 1943). -Sachalin austr.: fl. Tirikorogawa, (M. Tatewaki & Y. Takahashi n. 22827 ♂, n. 22829 ♂, n. 22826 ♀, n. 22822 ♀, n. 22824 ♂ 25 Jun. 1936).

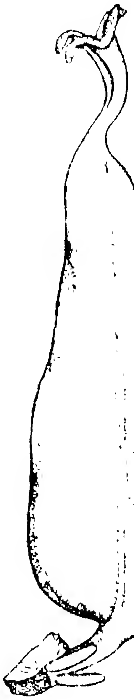

Fig. 2. *Toisusu cardiophylla* Kimura. Capsula cum glandulis, ex Tatewaki & Takahashi n. 22826. ×11.5.

Haec species a *Toisusu Urbaniana* Kimura bene differt foliis adultis minoribus, ovatis vel ovato-ellipticis vel ellipticis (nec oblongo-ellipticis nec oblongo-lanceolatis), tenuiter chartaceis (nec coriaceis), utrinque semper glaberrimis, apice subito acuminatis vel acutis (nec gradatim longe attenuato-acuminatis), basi plerumque cordatis, margine densius et saepe subhamulose serrulatis (nec grosse crenato-denticulatis), 1.6–2.0-plo (nec 2.5–3-plo) longioribus quam latioribus, amentis ♂ minoribus, glandulis florum ♀ oblongis vel linearibus (nec globosis), capsulis demum valde reflexis et ceteris notis.

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[1] Hic gratias ago cl. Dr. M. Tatewaki, Professori Botanicae Hokkaido Universitate Sapporensi, qui mihi summa liberalitate specimina haec pretiosa largitus est.

119) **Salix subfragilis** Andersson in Mem. Am. Acad. Arts Sci. n. ser. VI. p. 450 (1859) (Gray, Bot. Jap.).—Black apud Hooker in Hodgson, A Residence at Nagasaki & Hakodate etc. p. 342 (1961); in Bonplandia X: 6, p. 96 (1862) (System. Verzeichnis aller seit Thunberg in Japan gesammelten Pflanzen).—Franchet & Savatier, Enum. Pl. Jap. II: 1, p. 502 (1879).—Seemen, Salic. Jap. p. 74 (1903).—Schneider in Sargent, Pl. Wilson. III. p. 179 (1916).—Kimura in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 452 (1934) (Miyabe & Kudo, Fl. Hokkaido & Saghal. IV.); in Acta Phytotax. & Geobot. XIII. p. 188 (1943).—Nemoto, Fl. Jap. Suppl. p. 115 (1936).—Honda, Nom. Pl. Jap. p. 45 (1939).

Syn. 1) *Salix nipponica* Franchet & Savatier, Enum. Pl. Jap. I. p. 495 (1875) nom.; II: 1, p. 502 (1876).

Salix triandra L. var. *nipponica* (Franch. & Sav.) Seemen, Salic. Jap. p. 27, t. 2, fig. E-J (1903).—Kimura in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 401 (1934) (Miyabe & Kudo, Fl. Hokkaido & Saghal. IV.).

Nom. Jap. *Tatiyanagi* Matsumura, Nippon Shokubutsumeii p. 170 (1884).

Hanc speciem quae nobis longe fuit obscuram cum *Salice nipponica* Franch. & Sav. certe esse identicam concludo; confer etiam Kimura in Acta Phytotax. & Geobot. XIII. p. 188 (1943).

120) **Salix Pet-susu** Kimura in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol. XII. p. 317 (1937) (Symbol. Iteolog. IV.).—Honda, Nom. Pl. Jap. p. 44 (1939).

Syn. 2) *Salix sp.* Matsumura, Cat. Pl. Herb. Coll. Sci. Imp. Univ. p. 182 (1886).

Salix viminalis (non Linnaeus) Sugiyama, Hokkaido-Zyumokusiryo n. 158 (1890).

Salix viminalis L. var. *yezoensis* Schneider in Sargent, Pl. Wilson. III. p. 158 (1916).

Salix Gmelini Pallas var. *yezoensis* Kimura in litt. ex Görz, Sched. ad fasc. I. Salic. Asiat. p. 16 (1913).

Salix yezoensis Kimura in Bot. Mag. Tokyo XLV. p. 28 (1931).

Salix serotina Pallas var. *yezoensis* Kimura ex Görz in Fedde, Rep. Sp. Nov. XXXVI. p. 26 (1934) (Görz, Sched. ad fasc. III. Salic. Asiat.) in nota ad *S. serotinam*.

Nom. Jap. *Yezono-kinuyanagi* Kimura in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 430 (1934) (Miyabe & Kudo, Fl. Hokkaido & Saghal. IV.)

Hab. in Japonia (Yezo & Honsyû maxime septentrionali), Sachalin.—Specimina notabilia propter distributionem: Honsyû.—Prov. Mutu: Uchimappe, (S. Murai n. 59 ♀ fr. 24 Maio 1930); Sinzyô, (S. Murai n. 10 ♀ fl. 8 Maio 1932, fol. 19 Aug. 1932; n. 91 st. 19 Aug. 1932.—A. Kimura n. 911 ♀ fl., n. 912 ♀ fl. 8 Maio 1937; n.

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1) De synonymis amplis confer Kimura in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 401 (1934).

2) De litteratura synonymorum reliqua confer Kimura op. cit. p. 317 (1937).

2773 st. 7 Aug. 1938); Ôhata, (S. Kitamura st. 26 Jun. 1940).—Prov. Ugo: Higasitate-mura, (M. Matuda n. 5 st. 18 Jun. 1939); Nanakura-mura, (G. Koie n. 40 st. 2 Jul. 1939); Akita, (G. Koie n. 43 gemm. 11 Feb. 1940, ♀ fl. 13 Apr. 1940, fol. 17 Oct. 1939; n 53 ♂, n. 54 ♀, n. 55 ♂, n. 56 ♂, n. 57 ♂, n. 59 ♂ fl. 13 Apr. 1940, fol. 6 Oct. 1940).—Prov. Rikutyû: prope Miyako, (A. Kimura n. 2889 st., n. 2890 st. 17 Oct. 1940).

Hucusque sita esse videtur finis distributionis australis hujus Salicis in locis supra citatis.

121) **Salix gracilistyloides** Kimura in Bot. Mag. Tokyo XL. p. 8 (1926).
mstr **synpolyandra**[1] Kimura mstr. nov.

Stamina plerumque 4–5, raro 2 vel 3, filamentis ad apicem usque vel alte connatis.—Descr. specim. original.:—*Amenta* ♂ praecocia oblongo-cylindrica 3.7–5.2 cm longa ad 2.2 cm crassa, sessilia vel subsessilia, basi cataphyllis sessilibus 2–3 oblongis vel elliptico-oblongis apice late acutis, supra glabris subtus villosis, ad 7×3 mm magnis, vulgo reflexis instructa. *Bracteolae* ovato-lanceolatae, apice acutae vel acuminato-acutae nigrescentes, medio rubrae, basi flavo viridescentes, utrinque villosae, 4–5 mm longae vix 1.5 mm latae *Glandula* una ventralis linearis, apice truncata, obcompressa, apicem versus incurvata, circiter 1.7 mm longa; *stamina* 4–5 raro 2 vel 3, filamentis glabris ad 10 mm longis, aut alte aut prorsus in unum validum apicem versus obcompressum connatis; antherae ante explicationem rubrae plus minusve oblique ovales circiter 1 mm longae. Cetera ut in typica.

Hab. in Japonia. Honsyû.—Prov. Settu: in paludibus graminosis montis Rokkosan. (A. Kimura n. 444 ♂ fl [typus mstr.] in Herb. A. Kimura, 13 Apr. 1926, fol. 11 Jun. 1926).

122) **Salix Nakamurana** Koidzumi in Bot. Mag. Tokyo XXVII. p. 26 (1913).
mstr. **multicapsularis** Kimura mstr. nov.

Descr. specim. original.—*Amenta* coaetanea in pedunculis folia perfecta 3–4 gerentibus e gemma laterali ramuli anniculi prodeuntia, oblongo-cylindrica 16 mm longa 8–9 mm crassa, rhachidibus sericeis. *Bracteolae* apice acutae deorsum oblongae, utrinque sericeo-villosae, dimidia superiore parte brunnescentes, basi pallidae, circiter 2.8 mm longae 1 mm latae, pleraeque 3-nervatae. *Glandula* una ventralis ovato-rectangularis, apice truncata interdum leviter emarginata, circiter 1 mm longa et ad basin vix 1 mm lata, haud raro utrobique vel solum latere altero laciniata, laciniis nunc brevibus obtusis nunc longis (0.8, 2, 2.5 mm

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[1] **Synpolyandra**, categoria nova Salicum monstrositatis.—Hanc propriam combinationem *synapsis* staminum et *auxesis* numeri eorum synpolyandram nominari propono.

vel ultra) filiformibus, inde saepius curvulis apice leviter capitatis vel dilatatis et papilliferis. *Ovaria* vulgo 2–4 sub singulis bracteolis, carpophyllis singulis formata, omnia libera vel quorum 2–3 nonnumquam ad basin vel alte connata, e basi ovata elongato-conica, pauci- vel sparse pilosa, 2–2.5 mm longa 0.5–0.6 mm crassa; carpophyllis margine vulgo complete commissis, raro partim dehiscentibus, stipitibus glabris, liberis vel connatis 0.5–0.6 mm longis; stylis glabris elongatis 1.2–1.5 mm vel ultra longis; *stigmatibus* profunde bilobatis, laciniis linearibus erecto-patentibus 0.5–1.0 mm longis. Cetera ut in typica.

Hab. in Japonia. Honsyû. Prov. Sinano: in regionibus alpinis montis Yatugatake, inter saxa et rupes, (A. KIMURA n. 1243 fl. [typus mstr.] in Herb A KIMURA, 10 Jul. 1926; n. 1244 fl. 10 Jul. 1926.)

123) **Salix subopposita** MIQUEL in Ann. Mus. Bot Lugd.-Bat. III. p. 28 1867
mstr. **biglandulosa** KIMURA mstr. nov.

Flores ♂ omnium amentorum glandulis duobus (ventrali et dorsali) instructi.— Descr. specim. original.— *Amenta* ♂ praecocia in ramulorum superioribus partibus dense disposita, erecto-patula, opposita vel alterna, globoso-ellipsoidalia, apice rotundata, densiflora, subsessilia, basi foliolis 2–3 oblongis interdum obliquis sessilibus, apice obtusissimis, supra glabris infra sericeis 2.5–5 mm longis 1.0–1.7 mm latis suffulta, 0.9–12.5 mm longa, 0.8–0.9 mm crassa, rhachidibus pubescentibus. *Bracteolae* obovatae concavae apice rotundatae ultra mediam partem saepe rubicundam nigrescentes, basin versus pallide flavo-viridescentes, antice utrinque pilis leviter flavescentibus villosae, basin versus breviter pubescentes, 2 mm longae 1.2 mm latae. *Glandulae* 2 luteae, ventralis anguste ovata apice truncata, 1.2–1.3 mm longa, dorsalis linearis apice rotundata, satis angustior, dimidio fere ventralis aequilonga vel minor. *Stamina* 2, filamentis liberis gracilibus ad 5.5 mm longis, dimidia inferiore parte sparse pilosis; antheris late orbicularibus 0.8–0.9 mm longis. Cetera ut in typica.

Hab. in Japonia. Kyûsyû.—Prov. Hizen: in siccis graminosis montis Tenzan, (A. KIMURA n. 1132 ♂ fl. [typus mstr.] in Herb. A. KIMURA, 25 Apr. 1926).

124) **Salix alopochroa** KIMURA[1] in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol XII. p. 100 (1937).
mstr. **androgyna** KIMURA mstr. nov.

Descr. specim. original.—*Amenta* praecocia, sessilia vel subsessilia, rhachidibus plerisque pilis albidis pubescentibus, basi pilis ferrugineis vel albidis barbatis,

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[1] *Salici vulpinae* ANDERSSONII est proxima, sed praecipue differt cataphyllis amentorum vulgo fere nullis vel satis minoribus, amentis masculinis brevioribus atque crassioribus, floribus masculinis majoribus.

foliolis squamosis 2–3 vel nullis, sessilibus ovatis, lanceolato-ovaits vel oblongis, apice obtusis vel acutis, margine minute serrulatis, 5.5×2.3, 4×2 mm etc. magnis, supra fere glabris, subtus pilis ferrugineis micantibusque dense tectis suffulta, masculina, feminea et androgyna in uno eodemque frutice; *masculina* elliptico- vel oblongo-cylindrica 1.6–2 cm longa 0.75–0.9 cm crassa, *feminea* elongato-cylindrica, 2.2–3.6 cm longa, 0.6–0.8 cm crassa, *androgyna* alia floribus ♂ plurimis et ♀ paucis (1.5–2.6 cm longa, 0.9 cm crassa) aut vice versa (3.1–4.4 cm longa, fere 0.7 cm crassa), alia apice ♀ et basi ♂ (2.5–4 cm longa; pars ♂fera 1.1–1.2 cm crassa ♀feram ad 0.8 cm crassam longitudine fere aequans vel multo superans) aut raro vice versa (pars ♂fera brevior), alia floribus utrisque sine ordine mixtis (2.7–4.8 cm longa). *Bracteolae* ovatae vel ovato-oblongae apice obtusissimae dimidia superiore parte nigrescentes, circiter 1.5 mm longae 0.8–0.9 mm latae, utrinque pilis ferrugineis vel interdum cum albis mixtis villoso-sericeae, raro parce sericeae. *Glandula* una ventralis late rectangularis apice truncata vel rotundato-truncata, circiter 0.4 mm longa. *Stamina* 2, filamentis liberis glabris ad 5 mm longis, antheris ovalibus. *Ovaria* glabra ex ovata basi conica circiter 3 mm, demum 4.5 mm longa; stipitibus glabris circiter 0.6 mm longis glandulas 1.5- vix 2-plo superantibus; stylis glabris 0.4–0.5 mm longis. *Stigmata* parva, bifida, laciniis erecto-patentibus. Cetera ut typica.

Hab. in Japonia. Honsyû. - Prov. Hôki : prope Akamatu, pede montis Daisen, (A. Kimura n. 133 fl. (typus mstr.) in Herb. A. Kimura, 20 Apr. 1924).

125) **Salix gracilistyla** Miquel[1] in Ann. Mus. Bot. Lugd. Bat. III. p. 26 (1867); Prol. Fl. Jap. p. 214 (1867).

var. **adscendens** Kimura var. nov.

Ramis ramulisque adscendentibus nec declinatis nec procumbentibus dignoscenda. Descr. specim. original.: *Frutex* 3 m altus, trunco cortice griseo obtecto, mox supra solum in ramos validos circiter 8–9 cm crassos dividitur. *Rami* cinerei adscendentes fere recti. *Ramuli* elongati rectiusculi adscendentes, hornotini virides cinereo-tomentosi basi glabrescentes, annotini olivaceo-virides glabri. *Folia recentissima* dense adpresse albo-villosa, sub vernatione convoluta, exqua relaxata margine infero leviter revoluta. *Folia adulta* chartaceo-coriacea interstitiis 1.4–2.2 cm longis dissita, oblonga medio latiora, apice acuta vel breviter acuminata, basi acuta, margine anguste reflexa praeter basin vulgo integerrimam crenato-serrulata, serraturis minute mucronulatis, in medio folii 4–5 pro 1 cm, supra saturate viridia infra glauca, utrinque sericea, supra demum glabrescentia, 7.7–

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[1] De litteratura ampla hujus nominis synonymorumque confer Kimura in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 438 (1934).

13 cm longa, 2–3.6 cm lata, 3–4-plo longiora quam latiora; costa pallida supra elevata minute pubescente, subtus sericea vehementer prominente; nervis primariis supra fere planis subtus prominentibus, leviter arcuatis superne flexuosis, utroque latere 16–18 a costa sub angulis 40°–50° divergentibus, secundariis crebris infra elevatis inter primarios subparallele transversis, intermediis 1–3. *Petioli* semiteretes ad 1.4 cm longi basin versus dilatati supra canaliculati subtus convexi velutino-pubescentes. *Stipulae* oblique ovatae vel oblique angusto-ovatae, apice obtusae vel obtusissimae, margine fere integrae, supra virides basi glanduliferae, infra glaucae, utrinque sericeae, 6–14.5 mm longae 3–8 mm latae. *Amenta* ♂ praecocia sessilia oblongo-cylindrica densiflora recta vel leviter curvula, apice obtusissima, 4–5 cm longa 1.1–1.2 cm crassa, basi interdum cataphyllis paucis squamosis viridibus ovato-oblongis sursum angustatis summo obtusis infra albo-villosis supra praeter apicem marginemque glabris infra albo-villosis 6.5 × 2, 6.7 × 2.5 mm etc. magnis suffulta. *Bracteolae* lanceolatae apice longe acuminatae 3.1–4.0 mm longae 0.8 mm latae, dimidia superiore parte nigrescentes medio rubrae basi flavo-viridescentes, utrinque pilis rectis albo-villosae. *Glandula* una ventralis flavo-viridis linearis apice truncata 1.1–1.4 mm longa. *Stamina* 2, filamentis glabris ad apicem usque connatis 4.2–5.3 mm longis; antherae rectangulo-ovales ante anthesin rubrae, effuso polline fuscescentes 0.8 mm longae.

Nom. Jap. *Tati-nekoyanagi* KIMURA nom. nov.

Hab. in Japonia. Honsyû. Prov. Rikuzen: Sendai. (A. KIMURA n. 3042 ♂ fl. [typus var.] 11 Mart. 1949 in Herb. A. KIMURA, fol. [typus fol.] 21 Sept. 1948).

126) × **Salix sendaica** KIMURA in Sci. Rep. Tôhoku Imp. Univ. 4 ser. Biol. VI. 2, p. 196 (1931) (Contr. Salic. Jap. IV.); in Jour. Fac. Agr. Hokkaido Imp. Univ. XXVI. 4, p. 439 (1934) (MIYABE & KUDO, Fl. Hokkaido & Saghal. IV.); in Hyôgoken-Tyûtô-kyôiku-Hakubutugakuzassi VII. p. 203 (1941). —NEMOTO, Fl. Jap. Suppl. p. 114 (1936). —HONDA, Nom. Pl. Jap. p. 44 (1939).

= *Salix Bakko* KIMURA × *S. vulpina* ANDERSSON.

Descr. specim. original. ♂: *Frutex* circiter 3 m altus. *Ramuli* teretes ascendentes, latere soli aperto purpureo-fusci, altero abscondito virides, in sicco atrobadii, hornotini in partibus juvenilibus minutissime crispo-pubescentes mox glabrescentes, annotini glaberrimi. *Rami* cinereo-virides. *Lignum nudum* vibicibus distinctis praeditum. *Cataphylla* sterilium ramulorum subsessilia vel brevissime petiolata, anguste ovato-elliptica, elliptico-oblonga, elliptico-lanceolata vel elliptica, utrinque obtusa, margine integerrima, supra glabra infra villosissima, 11–17 mm longa 4–6 mm lata, superiora majora. *Folia recentissima* sub vernatione convoluta, ex qua relaxata margine infero revoluta, utrinque tomentosa; *adulta* chartacea interstitiis 1.3–5 cm longis dissita, oblonga vel elliptico-oblonga vel elliptica, plerumque medio latiora, apice acuminata raro acuta, basi margine leviter convexo vel rectiusculo acuta ad obtusissima, margine subundulatim crenato-denticulata, dentibus in medio folii 2–4 pro 1 cm, semper mucronulatis, interdum satis obsoletis subintegra, supra glabra saturate viridia impressi-nervata, subtus glaucina pilis curvulis pubescentia, 9–18 cm longa, 3.8–6.8 cm lata, 2.3–3-plo longiora quam latiora, inferiora minora; costa supra leviter convexa demum glabrescente, subtus vehementer prominente pilis curvulis albis saepe cum ferrugineis mixtis tomentella; nervis primariis utrinsecus 9–12, arcuato-ascendentibus ante marginem flexuosis in medio folii a costa sub angulis 60°–80°, prope basin 30°–50° proficiscentibus, supra glabris immersis infra prominentibus tomentellis; secundariis supra impressis infra elevatis aut crebris subregularibus aut fere irregularibus; intermediis 1–3. *Petioli* semiteretes pubescentes infra demum glabrescentes 1.4–2.4 cm longi. *Stipulae* oblique ovatae vel semicordatae acutae denticulatae, utrinque glabrae vel subtus pubescentes, supra virides infra glaucinae, 5×3, 10×5.5, 15×9, 20×9 mm etc. magnae. *Amenta* ♂ praecocia densiflora, oblongo-cylindrica apice obtusissima, saepe sursum paullo angustiora, recta vel leviter curvula, 2.8–5 cm longa 1.1–1.5 cm crassa, rhachidibus villosis, pedunculis villosis fere ad 9 mm longis, cataphyllis 4–5 forma magnitudineque iis sterilium ramulorum simillimis instructis. *Bracteolae* oblongae apice obtuse acutae, dimidia superiore brunneae ceterum pallidae, utrinque albo-villosae, 2.4–2.5 mm longae 0.9–1.0 mm latae. *Glandula* una ventralis trapeziformis fere 0.5 mm longa 0.3 mm lata. *Stamina* 2, filamentis liberis ima basi paucipilosis ceterum glabris, 5.5–6 mm longis; antherae luteae ovales 0.8 mm longae.

Nom. Jap. *Sendai-yanagi*.

Hab. in Japonia. Honsyû.—Prov. Rikuzen: prope Sendai, (A. KIMURA n. 2302 ♂ fl. [typus ♂] 11 Apr. 1940, in Herb. A. KIMURA fol. [typus fol.] 13 Jul. 1939, fl. 28 Apr. 1934).

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ON THE SECRETION OF FERTILIZIN IN THE EGGS OF A SEA URCHIN, *STRONGYLOCENTROTUS PULCHERRIMUS* (A. AGASSIZ)*)

By

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F.R. LILLIE (1913) has advanced an interesting hypothesis that the fertilizable condition of the egg is due to the presence of a soluble colloidal substance, fertilizin, which forms a chemical link between the egg and sperm. This substance is characterized by its aggulutinating effect upon the sperm, and is readily detected in egg water. He has demonstrated the production of fertilizin from ripe unfertilized eggs of *Arbacia* and of *Nereis*, and has shown that this substance is not produced by the fertilized egg. LOEB (1914) suggested, on the other hand, that the agglutinating stbstance is merely the dissolved jelly that surrounds the egg. Recently, TYLER (1941, 1948) expressed the opinion that fertilizin is a component of the jelly layer and is not secreted by the ripe eggs.

In the present paper it is intended to give the results of observations on the mode of secretion of fertilizin by the unfertilized and by the fertilized eggs of a sea urchin, *Strongylocentrotus pulcherrimus* (A. AGASSIZ).

MATERIAL AND METHODS

The material used was a sea urchin, *Strongylocentrotus pulcherrimus* (A. AGASSIZ), from Matusima and Asamusi. For the titration of the agglutinating power of fertilizin two methods were used. The one is the drop method, in which 2 drops of sperm suspension were mixed with 2 drops of testing fluid on the depression slide, and then the mixture were observed under the microscope. The other is a modificiation of the ring method. A drop of sperm suspension on a slide is covered with a cover slip, and then a drop of testing fluid is sucked in from a side of the cover. The agglutinating reaction is observed at the boundary of the two fluids. The former method was ten times sensitive than the latter, but the latter was more accurate.

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*) Contributions from the Marine Biological Station, Asamushi, Aomoriken. No. 185.
These researches were aided by the Scientific Research Expenditure of the Department of Education.

SECRETION OF FERTILIZIN BY THE JELLY-LESS UNFERTILIZED EGG

Whether the jelly-less unfertilized egg can secrete the fertilizin or not were tested. The jelly coat of the unfertilized egg was removed by placing for 30 minutes in sea water of pH 4.0 to 4.8 acidulated with HCl, butyric acid, tartaric acid, citric acid, or with succinic acid. Supermatants were removed by centrifuging the egg suspensions. The eggs were then placed for 20 hours in sea water of pH 8.2. The concentration of fertilizin of the jelly solutions as well as of the subsequent sea water extracts were determined by the drop method.

Table I Secretion of fertilizin in sea water by the jelly-less unfertilized eggs

Jelly was removed with acidic sea water for 30 minute, and after this the eggs were placed in sea water for 20 hours. Eggs volume 6.6 cc each. Volume of fluid 14cc Titration of units with the drop method.

No. of exp.	Jelly-fertilizin			Units of cytofertilizin secreted into sea water within 20 hrs	Total
	Acids	pH	Units		
1	HCl	4.0	256	512	768
2	Butyric	4.4	1024	64	1088
3	Tartaric	4.0	512	512	1024
4	Citric	4.2	1024	128	1152
5	Succinic	4.8	1024	256	1280

The results are shown in Table I The jelly solutions showed high concentrations of fertilizin of 256 to 1024 units. The sea water extracts of the jelly less unfertilized eggs also showed high concentrations of 64 to 512 units. The value was much higer than the expected error. It reveals that the jelly-less unfertilized egg secreted the fertilizin into sea water.

The above result shows that the fertilizin is not only a component of the jelly but also an inclusion of the unfertilized egg. If so, the total amount of the fertilizin including the jelly and egg cell will be larger than that of the jelly fertilizin. A batch of unfertilized eggs were divided equally into eight portions. Six of them were placed in acidic sea water acidulated with various acids. A portion was centrifuged for removing the jelly. The last one portion was boiled for one minute in sea water. The concentrations of fertilizin of those extracts were measured by the modified ring method. The result was that a large amount of fertilizin was extracted by boiling sea water in comparison with other methods (Table II).

Table II. Comparison of the amount of fertilizin extracted by acidic sea water and by boiling

Eggs volume 0.5 cc each. Fluid volume 14cc. Acidic sea water was adjusted to pH 4.5. Titration with the modified ring method.

No. of exp.	Methods	Units
1	HCl for 20 minutes	32
2	Lactic acid 〃	32
3	Butyric acid 〃	32
4	Citric acid 〃	32
5	Tartaric acid 〃	32
6	Succinic acid 〃	32
7	Centrifuging for 2 minutes, 1000 rev/min.	32
8	Sea water boiling for 1 minute.	128

SOLUBILITY OF CYTOFERTILIZIN

In the above mentioned observation it was suggested that the jelly-less unfertilized eggs secrete the fertilizin into sea water. This portion of the fertilizin will be tentatively called the cytofertilizin.

The solubility of the cytofertilizin in sea water as well as in 1 Mol solution of urea of various pH was tested. A batch of unfertilized eggs were divided into three portions. Two of them were centrifuged to remove the jelly, and then, boiled for one minute in sea water of pH 8.3 and 4.5 respectively. A strong agglutinating reaction was detected in the former, while it was negative in the latter. The last one third was placed in HCl-sea water of pH 4.5 to remove the jelly, and then boiled in sea water of pH 8.3. This showed also a

Table III. Solubility of cytofertilizin in sea water of various pH

Eggs volume 0.5 cc. each. Fluid volume 14cc. Titration with modified ring method.

No. of exp.	Jelly-fertilizin		Cytofertilizin		Total
	Method	Units	Method	Units	
1	Centrifuged for 2 minutes, 1000 rev. /min.	64	Jelly-less eggs were boiled in sea water pH 8.3	32	96
2	Centrifuged for 2 minutes, 1000 rev. /min.	64	Jelly-less eggs were boiled in sea water pH 4.5	0	64
3	HCl-sea water of pH 4.5 for 10 minutes	32	Jelly-less eggs were boiled in sea water pH 8.3	16	48

strong agglutinating reaction. (Table III). It is concluded from those observations that the cytofertilizin is more soluble in alkaline sea water than in acidic sea water.

The same observation was carried out with non-electrolite, 1 Mol urea solution. A batch of unfertilized eggs was divided into three equal portions, and then centrifuged to remove the jelly. They were then placed for a minute in urea solutions of pH 8.3, 7.0 and of 4.5 respectively. After this, the acidic as well as the neutral urea solutions were adjusted to pH 8.3, and then the title of the fertilizin of those three solutions were determined. The result was that the alkaline urea solution dissolves more fertilizin than the acidic. (Table IV).

Table IV. Solubility of cytofertilizin in 1 Mol urea solutions of various pH

Jelly-less eggs were washed for 1 minute in 1 Mol urea solutions. Eggs volume 0.5 cc. each. Volume of fluid 14 cc.

No. of exp	Units of jelly-fertilizin centrifuged for 2 min. in 1000 rev./min.	Cytofertilizin in urea solutions	
		pH	Units
1	64	8.3	16
2	64	7.0	2
3	64	4.5	0

Those two observations agree in the point that the solubility of the cytofertilizin is large in alkaline solutions. Since Loeb's discovery acidic sea water was mostly used for dissolving the fertilizin contained in the jelly. My result opposes this and shows that the solubility of the cytofertilizin is large in alkaline solutions. Accordingly, the fertilizin must be distinguished from the jelly coat.

SECRETION OF FERTILIZIN BY FERTILIZED EGGS

According to Lillie's hypothesis the fertilizin in the egg will be inactivated in the fertilized egg by the antifertilizin. This will be the case as the fertilized egg does not secrete the fertilizin. And it is difficult to detect the fertilizin in the fertilized egg, unless some treatment to prevent the inactivation of fertilizin is done.

Fortunately, the writer could detect the fertilizin in the fertilized eggs, which were previously treated with HCl-sea water. The jelly coat of the unfertilized egg was removed with acidic sea water. This procedure has an important effect to retain the fertilizin in the fertilized egg, and also to prevent the toughening of the fertilization membrane, on which the writer will describe in another paper (Motomura 1950). The jelly-less eggs were fertilized in sea water and after three minutes they were shaken in a test tube with half-filled sea water to remove the fertilization membrane. The supernatant containing the perivitelline fluid showed a remarkable reaction of fertilizin (Table V).

Table V. Secretion of cytofertilizin into the perivitelline space of the fertilized eggs

Series of treatment / No. of exp.	1	2	3
Unfertilized egg 5 min. in HCl-sea water, pH 4.5	32 units	64 units	256 units
↓			
Unfertilized egg 5 min in HCl-sea water, pH 4.5	0	0	1
↓			
Unfertilized egg 1 min in sea water	0	0	2
↓			
Eggs were fertilized in sea water, and after 3 min were shaken in a test tabe with sea water	4	16	8

It was ascertained in the next experiment that the fertilizin in the perivitelline space of the fertilized egg which has been treated with HCl-sea water prior to fertilization is not inactivated for more than thirty minutes (Table VI). The jelly coat of the unfertilized eggs was removed by washing three times with HCl-sea water. The eggs were then fertilized in sea water, and were placed therein for thirty minutes, the sea water was changed every fifteen minutes. Lastly, the eggs were shaken in a test tube with sea water to break the fertilization membrane. The fertilizin was detected in the last mentioned fluid.

Table VI. Secretion of cytofertilizin into the perivitelline space of the fertilized eggs (Eggs vol. 0.5 cc., Fluid vol. 14 cc)

Series of treatment	Units
Unfertilized eggs were washed in HCl sea water, 1 min.	64
↓	
Unfertilized eggs were washed in HCl sea water, 1 min	32
↓	
Unfertilized eggs were washed in HCl sea water, 1 min.	4
↓	
Fertilized in sea water, and placed in it for 15 min	0
↓	
Sea water was changed, and placed in it for 15 min	2
↓	
Fertilization membrane was broken by shaking in a test tube with sea water.	16
↓	
Menbrane-less fertilized eggs were bioled in sea water	0

Those results show that the cytofertilizin is discharged into the perivitelline fluid when the egg is fertilized.

DISCUSSION

The old problem whether the fertilizin is secreted by the sea urchin egg has been discussed by many authorities. Recent investigators, Tyler (1941, 1948) and Cornmann (1941), have the opinion that the fertilizin is a component of the jelly layer and is not secreted by the ripe egg. The writers results agree with Lillie's in the point that the jelly-less unfertilized egg secretes the fertilizin in sea water. But, as to the solubility the cytofertilizin differs remarkably from the jelly coat. And from this fact it is concluded that acidic sea water is not suitable for dissolving the cytofertilizin.

The fertilizin of the eggs of *Strongylocentrotus pulcherrimus* was easily extracted by a short boiling in sea water. Although it is said that the fertilizin is destructed by heat, Lillie's observation showed its thermostability in some extent; that is, the fertilizin of *Arbacia* is not destructed by heating over 90°C for five minutes. The writer's observation do not contradict Lillie's results with *Arbacia*.

According to Lillie the fertilizin is not produced by fertilized egg, because it is inactivated by the antifertilizin of the egg soon after fertilization As mentioned above the present writer showed that fertilizin can be detected in the perivitelline fluid of the fertilized eggs, when they were placed for a while in acidic sea water prior of fertilization. This shows that the antifertilizin was lost by the treatment with acidic sea water. And, simultaneously, the eggs lost their capacity of toughening the fertilization membrane. From those facts it is probable that fertilizin and antifertilizin have some chemical relations with the substances of the fertilization membrane. Motomura (1941) showed that the fertilization membrane of sea urchins become hard by secreting the Janus Green granules, cortical granules, into the perivitelline space. But according to the writer's present observation those granules are not discharged in acidic sea water. Recently another new factor for the toughening of fertilization membrane was discovered. This factor, the third factor which will be described in another paper, is extracted with acidic sea water from the unfertilized egg of *Strongylocentrotus pulcherrimus* and from the fertilized eggs of *Temnopleurus hardwickii* and of *Clypeaster japonicus* immediately after fertilization. The problem is, therefore, whether the third factor is identical with the antifertilizin of the egg.

SUMMARY

The nature of fertilizin in the eggs of a sea urchin, *Strongylocentrotus pulcherrimus* (A. Agassiz) was described. The fertilizin is secreted by the jelly-less

unfertilized egg, in which the jelly coat was removed by sea water acidulated with HCl, butyric, tartaric, cytric or succinic acids in pH 4.0 to 4.8. Fertilizin was extracted by boiling sea water for a minute. The solubility of cytofertilizin in sea water as well as in isotonic solution of urea is higher in alkaline than in acidic media. And, consequently, the cytofertilizin is distinguished from the jelly coat. The fertilizin was detected in the perivitelline fluid of the fertilized egg which was placed in acidic sea water before it is fertilized. In this case it was remarkable that the fertilizin was not inactivated and that the fertilization membrane did not become tough.

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ON A NEW FACTOR FOR THE TOUGHENING OF THE FERTILIZATION MEMBRANE OF SEA URCHINS*)

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(With 2 Text-figures)

In 1941 MOTOMURA showed that the fertilization membrane is composed of two kinds of materials, the vitelline membrane and the Janus green granules. The former is elevated from the egg surface by the colloid osmotic pressure of the secreted portion of the Janus green granules, and the latter from the precipitation membrane at the inner surface of the elevated vitelline membrane by the presence of calcium ions. In 1947 RUNNSTRÖM also discovered independently those granules, the cortical granules, in the cortical layer of the eggs of sea urchins and described the function of them for the membrane formation.

Recently the writer took note of another or the third factor which is soluble in acidic sea water, for toughening of the fertilization membrane in addition to the above mentioned factors The importance of the third factor on the formation of the fertilization membrane will be described below

The observations were carried out on the eggs of *Strongylocentrotus pulcherrimus* (A. AGASSIZ) and *Temnopleurus hardwickii* (GRAY) at the Asamushi and of *Clypeaster japonicus* DÖDERLEIN at the Misaki Marine Biological Stations.

My sincere thanks are due to Dr. K. DAN, Professor of Tokyoto University, to Mrs. Dr J.C. DAN and to Mr. Y ENDO for working facilities and for the generous supply of materials.

OBSERVATION

I. *STRONGYLOCENTROTUS PULCHERRIMUS*

In the course of observations on the fertilizin of this species, the writer noticed an interesting phenomenon that when the egg was washed for 10 minutes with HCl-sea water prior to fertilization, it forms a thin membrane by fertilization without toughening as in the normal case. The fertilization membrane thus formed could be torn by shaking. This was accertained more quantitatively.

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*) Contributions from the Marine Biological Station, Asamushi, Aomoriken, No. 186.
These researches were aided by the Scientific Research Expenditure of the Department of Education.

The experiment was carried out in two ways. The one is that the unfertilized eggs were centrifuged to remove the jelly, and next, placed for 10 minutes in HCl-sea water of pH 4.5, and then fertilized in normal sea water. In the other set the extract of the unfertilized eggs were used. The unfertilized eggs were centrifuged to remove the jelly, and then extracted for 10 minutes with HCl-sea water of pH 4.5. This extract was adjusted to pH 8.3 before use. The jelly-less eggs were washed with HCl-sea water and then fertilized in this extract. Three hours after fertilization the toughness of the membrane was tested by shaking the eggs in a test tube with half-filled fluids. The fertilization membrane in the latter case was as tough as the normal egg, while in the former case it was thin and could easily be torn (Table I).

Table I. Inhibition of toughening of the fertilization membrane by treating the unfertilized eggs with HCl-sea water in *Strongylocentrotue pulcherrims*

The eggs were shaken with sea water in a test tube 30 minutes after fertilization.

Treatment	Toughness of fertilization membrane		Total number of eggs
	Broken	Complete	
Washed with HCl-sea water, fertilized, kept in sea water	31	64	95
Washed with HCl-sea water, fertilized, and kept in neutralized egg water	4	80	84
Normal fertilized eggs.	5	65	70

Those facts show that by washing the unfertilized egg with acidic sea water a factor necessary for the toughening of the fertilization membrane was dissolved away. This factor can be added again to toughen the membrane.

The chemical nature of this factor is not yet clear. This factor will be assumed to be a basic substance because it is soluble in acid and not in sea water. It is distinguished from the Janus green granule in that the latter is not secreted in the acidic sea water. It is contained in the egg cytoplasm, and is different from the jelly. The reason is that the extract of the jelly-less egg contains this factor, if the jelly was proviously removed by centrifuging. The writer assumes, therefore, that this factor is new, and he tentatively calls it the third factor of toughening the fertilization membrane.

II. *CLYPEASTER JAPONICUS*

A) *Effect of the Jelly on the Toughening the Fertilization Membrane.* The jelly of the unfertilized egg was first removed by washing with HCl-sea water of pH 5.0 for 10 minutes. The eggs were then fertilized in sea water. Tough membrane as in the normal egg was formed. Thus, the jelly of the eggs of Clypeaster showed no effect on the toughening the fertilization membrane.

B) *Deflation of the Fertilization Membrane in HCl-Sea Water and Inflation in Sea-Water.* The fertilization membrane deflates when the fertilized egg is put into HCl-sea water before the toughening of the membrane. The experiments were as follows. The jelly of the unfertilized egg was removed by washing for 10 minutes in HCl sea water of pH 4.5. The eggs were fertilized in sea water, and after one and a half to two minutes, when the fertilization membrane began to elevate, they were put into HCl-sea water of pH 4.5. The fertilization membrane of those eggs soon began to deflate, and the perivitelline space became invisible.

Those eggs can inflate the membrane again, if they are transferred to sea water. This shows that the inflation is caused by a colloidal substance which is blocked to swell in acidic sea water, because it was ascertained by the method of vital staining that the Janus green granule, a colloidal pre-membrane stuff, had been already discharged from the cortical layer.

The swelling of the colloidal substance is destroyed by placing for a long time in acidic sea water. The eggs placed for one hour in acidic sea water showed a good inflation of the membrane, but after two hours it was not the case.

C) *The Third Factor of the Toughening of the Fertilization Membrane.* The toughness of the fertilization membrane decreases by the treatment with acidic sea water. Although the membrane inflates again, if the fertilized eggs are transferred from acidic sea water to normal sea water, it soon begins to deflate again even in sea water. Those phenomena are shown in Fig. 1. The jellyless eggs were fertilized in sea water and were transferred within one and a half minutes into HCl-sea water of pH 5.0. After 30 minutes they were transferred again into sea water. The changes of diameter of the ferti-

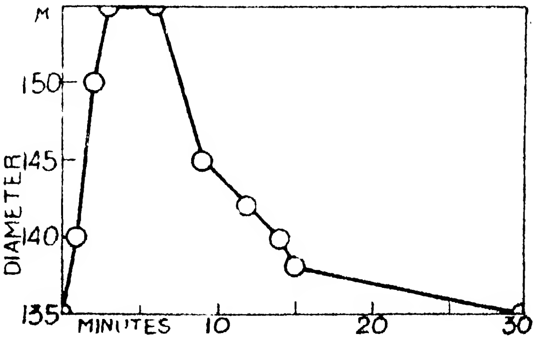

Fig. 1. Inflation and subsequent deflation of the fertilization membrane in sea water in the fertilized egg treated with acidic sea water. *Clypeaster japonicus.*

lization membrane were measured. The membrane at first began to inflate up to 155 μ. and from 6 minutes onward it sank again After 30 minutes no perivitelline space was observed. Those facts show that the fertilization membrane of the acid-treated egg is thin and elastic in comparison with the normal one, and that the third factor, a factor necessary for the toughening the fertilization membrane, was lost by this treatment.

D) *Toughening the Fertilization Membrane by Adding the Extract.* Likewise in the case of *Strongylocentrotus pulcherrimus* the third factor was detected in the acidic extract, which was prepared by washing the jelly-less fertilized egg with HCl-sea water of pH 4.5 for 30 minutes and was neutralized to pH 8.3 The fertilized eggs with deflated membrane in HCl-sea water was divided into two portions, which were put into the extract and normal sea water respectively. In both cases the membrane elevates at the beginning, but after this it sank in sea water, while it became tough and did not sink again in the extract. (Table II). From those observations the writer believes that this factor is identical in its function with that of *Strongylocentrotus pulcherrimus*.

Table II. Toughening of the fertilization membrane by the egg extract in the fertilized eggs of *Clypeaster japonicus*

Fluids	No. of eggs with deflated fertilization membrane	No. of eggs with complete fertilization membrane	Total
Acid treated eggs were placed in the egg extract	1	60	61
Acid treated eggs were placed in sea water	150	1	151

E) *The Time of Discharge of the Third Factor* Contrary to *St. pulcherrimus* the third factor is difficult to extract from the unfertilized egg of *Clypeaster*. This was ascertained as follows. The extract of the unfertilized egg with HCl-sea water was compared with that of the fertilized egg. One and a half minutes after fertilization the eggs were put into HCl-sea water of pH 4.5 for 30 minutes, and after this they were transferred in the following three fluids; 1) extract of the unfertilized eggs by HCl-sea water, 2) extract of the fertilized egg as mentioned in D), 3) sea water. As the extracts in 1) and in 2) were acidic, they were neutralized to pH 8.3 before use. After one hour the eggs with the elevated as well as the sunken membrane were counted (Table III).

As the results it was remarkable that only the extract of the fertilized egg was effective for toughening the membrane. Those results showed that in the eggs of *Clypeaster* the third factor is present besides the Janus green granules and calcium ions, and that the third factor of this species is not secreted before the fertilization.

Table III. Comparison of the effect of egg extracts of unfertilized and of fertilized eggs on the toughening of the fertilization membrane in *Clypeaster japonicus*. Egg volume 0.5 cc each.

Fluids	No. of eggs with deflated fertilization membrane	No. of eggs with complete fertilization membrane	Total
Extract of unfertilized eggs	77	8	85
Extract of fertilized eggs	10	80	90
sea water	100		100

III. *TEMNOPLEURUS HARDWICKII*

A) *The Third Factor is not Secreted by the Unfertilized Egg.* The unfertilized eggs of *Temnopleurus* was placed in acidic sea water of pH 4.5 to 5 for 30 minutes and then fertilized in sea water. The normal fertilization membrane wsa formed in all cases. The factor for toughening of the membrane was not lost by the treatment. The loss of the jelly coat exhibits no effect on the membrane formation.

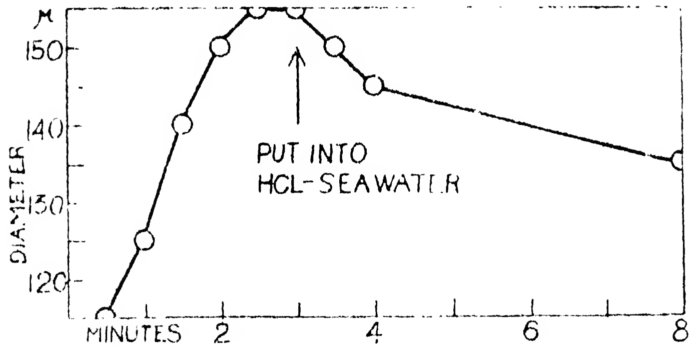

Fig. 2. Elevation of the fertilization membrane of a normal egg, and deflation of the membrane after placing in acidic sea water. *Temnopleurus hardwickii.*

B) ***The Deflation and Inflation of the Fertilization Membrane.*** The fertilization membrane elevated to the largest diameter of 155 μ three minutes after fertilization. The thickness of the perivitelline space measured 40 μ at this time. When the fertilized eggs were put into HCl-sea water of pH 4.5 three minutes after fertilization, the membrane deflates gradually to $135\,\mu$, and after this it remained in this position (Fig. 2). If the eggs were transfered to sea water again, the membrane elevated up to the maximum diameter of the normal fertilization membrane. In this species, therefore, the mode of deflation of the fertilization membrane in the acidic as well as of inflation in the alkaline media is quite similar to that of *Clypeaster*, except that the deflation is incomplete.

C) *Secretion of the Third Factor from the Fertilized Egg.* Three minutes after fertilization the eggs were washed for 10 minutes in HCl-sea water of pH 5.0, and then were transferred to normal sea water. The fertilization membrane elevated again in the last fluid. Thirty minutes after the stay in sea water the toughness of the membrane was compared with that of the normal egg (Table IV).

The membrane of the eggs treated with acidic sea water was easily torn by centrifuging for a minute with 2100 revolutions, while in the normal egg it remained unaffected. Those facts shows that the third factor was lost by treating the fertilized eggs with HCl-sea water. It was also noticed that the toughness of the membrane approached to the normal when the eggs were treated too early with the acidic sea water. That is, when the eggs were transferred 2 minutes after fertilization into acidic sea water only a half of the membrane was torn by the same centrifugal force (Table IV).

Table IV. Loss of the factor for toughening the fertilization membrane in *Temnopleurus* by washing the fertilized eggs with HCl-sea water

The eggs were placed for 10 minutes in HCl-sea water of pH 5.0 from 2 to 3 minutes after fertilization, and then replaced in sea-water. Control showed normal fertilized eggs. Membrane was tested by centrifugral force of 2100 revolution.

Treatments	No. of eggs with torn membrane	No. of eggs with complete membrane	Total
Put into HCl-sea water 2 min. after fertilization.	179	168	347
Put into HCl-sea water 3 min. after fertilization.	112	7	119
Control	0	301	301

D) *The Time of Secretion of the Third Factor.* In the preceding section the writer pointed out that the secretion of the third factor is remarkable at 3 minutes after fertilization than before. Serial experiments to ascertain the time of the maximum secretion showed that it was maximum in the eggs transferred to the acidic sea water 5 minutes after fertilization, because the least toughness was observed in this case (Table V). The results show, therefore, that the secretion of the third factor reaches the maximum within 3 to 5 minutes after fertilization.

Table V. Time of secretion of the third factor by the fertilized eggs of *Temnopleurus hardwickii*

The method of treatment was the same as in Table IV.

Treatments. Time elasped before putting into HCl-sea water after fertilization.	No. of eggs with torn membrane	No. of eggs with complete membrane	Total
1 minute	55	86	141
3 minutes	72	136	208
5 minutes	287	23	310
10 minutes	0	118	118

E) *Toughening Effect of the Third Factor*. Three minutes after fertilization the eggs were extracted for 10 minutes with HCl-sea water of pH 5.0. The eggs were divided into two lots. One lot was transferred to the ordinary sea water, and the other lot was put into the above mentioned extract which was neutralized to pH 8.3 before use. After placing in those fluids for 30 minutes, the eggs were centrifuged for two minutes at 2200 revolutions per minute. In the normal sea water the fertilization membrane was torn in most of the eggs, and on the contrary, in the egg extract the membrane remained normal. This shows that the fertilization membrane was toughened in the egg extract (Table VI).

Table VI. Toughening effect of the third factor for the fertilization membrane in *Temnopleurus hardwickii*.

The fertilized eggs were washed with HCl-sea water and then placed either in sea water or in the extract. Control is normal eggs. After 30 minutes the eggs were centrifuged.

Treatments	No. of eggs with torn membrane	No. of eggs with complete membrane	Total
Treated with acid and then placed in sea water	876	183	1059
Treated with acid and then placed in the extract.	182	536	718
Control	0	250	250

DISCUSSION

In the previous paper (MOTOMURA 1941) the writer showed in the eggs of ***Strongylocentrotus pulcherrimus*** that the vitelline membrane is elevated by the secretion of a colloidal substance, the Janus green granules, into the perivitelline space, and that the formation of the fertilization membrane is performed by precipitating those granules inside the vitelline membrane in combination with calcium ions. The Janus green granules are observed not only in the eggs of ***Strongylocentrotus*** but also in that of *Temnopleurus* and of *Clypeaster*. And, according to MOORE (1933) the mechanism of the membrane formation is said to be smilar, because the premembrane stuff of those eggs are equally dissolved by isotonic alkaline solution of urea. It was already pointed out by the writer that the Janus green granules is more soluble in alkaline than in acidic solutions (1941). The third factor described in this paper is, therefore, different from the Janus green granules in its solubility. It is different from calcium ions, because all of the experiments were carried out in the presence of calcium ions. The chemical nature of the third factor was not determined. The solubility shows its nature to be of a basic substance. The factor diffuses out of the vitelline membrane in acidic sea water, and comes in again through the elevated vitelline membrane. And as far as the writer is aware the third factor concerning the toughening the fertilization membrane has never been reported although the cortical granules, the Janus green granules, and calcium ions were previously known.

A large numbers of the egg secretions hitherto reported are concerned with the fertilizin. The fertilizin of the eggs of *Strongylocentrotus pulcherrimus* is of a different nature as reported in another paper (MOTOMURA 1950). It was hardly detectable in *Temnopleurus* and in *Clypeaster*. TYLER and O'MELVENY (1941) described an acid-soluble substance, antifertilizin, which is extracted by acidic sea water of pH 3.5 to 6.0 from the sperm of a sea urchin. The antifertilizin has been also reported by RUNNSTRÖM (1935) from the egg, and by FRANK (1939) from the sperm of *Arbacia*. If the nature of antifertilizin reported by those authorities is identical, the factor described in this paper is mostly related to the antifertilizin in its solubility to acid. But the writer has not yet examined the reaction of the third factor to the antifertilizin.

The time of release of the third factor differs according to the species. In *St. pulcherrimus* it is easily extractable from the unfertilized egg, while in *Temnopleurus* and in *Clypeaster* it is released only in a short time after fertilization. This suggests that the third factor is combined in the unfertilized egg in the last mentioned two species. From literatures, however, the writer was aware that in many species of sea urchins the fertilization membrane is not affected by treating the unfertilized egg with acidic sea water, as in *Arbacia* (LILLIE, 1914), *Strongylocentrotus purpuratus* (LOEB, 1914), *Echinarachnius* (JUST, 1919), and in *Paracentrotus*, *Sphaerechinus* and *Psammechinus* (HULTAN, 1948). The combined state of the third factor in the unfertilized eggs may be common in many species of sea urchins.

As to the mode of reaction of the third factor the writer has not yet studied the problem thoroughly. The factor is secreted into the perivitelline space following the secretion of the Janus green granules. The reason is that the membrane is not toughened at the beginning of secretion of the Janus green granules, and that the factor is extracted effectively from three minutes after fertilization onward, when the Janus green granule has already been discharged in *Temnopleurus* and in *Clypeaster*. Whether the third factor is an enzym or not will be varified in the next experiment. The writer is inclined to believe that the third factor is secreted by the egg immediately after fertilization into the perivitelline space accompanied with the Janus green granule, and that it takes a part in the toughening of the fertilization membrane with the other two factors, the Janus green granules and calcium ions.

SUMMARY

A new factor, the third factor, for the toughening of the fertilization membrane was described in the eggs of the sea urchins, *Strongylocentrotus pulcherrimus*, *Temnopleurus hardwickii* and *Clypeaster japonicus*. The third factor is secreted by the egg immediately after fertilization into the perivitelline space, and takes part in the toughening of the fertilization membrane with the other two factors, the Janus green granules and calcium ions. The third factor is extracted by acidic sea water in unfertilized eggs of *Strongylocentrotus pulcherrimus*, and in fertilized eggs of *Temnopleurus hardwickii* and of *Clypeaster japonicus*. By losing the third factor the toughening of the fertilization membrane does not occur. By adding this factor again to the eggs the fertilization membrane become tough.

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